

DETERMINANTS OF SEVERITY AND PROGRESSION OF DIABETIC RETINOPATHY IN SOUTHERN MALAWI

**Thesis submitted in accordance with the requirements of the University of
Liverpool for the degree of Doctor in Philosophy**

Philip Ian Burgess



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STATEMENT OF ORIGINALITY

I declare that this thesis was composed by me and that the work contained therein is my own, except where explicitly stated otherwise in the text. The work within this thesis has not been submitted for any other degree or professional qualification.

ABSTRACT

Background Sub-Saharan Africa faces an epidemic of diabetes. The prevalence and incidence of sight threatening diabetic retinopathy in developed countries and associations between systemic factors, including glycaemic control, blood pressure and blood lipid levels, are well documented. In contrast the epidemiological literature from sub-Saharan Africa is sparse. In this resource-poor setting, population-specific variables such as a high burden of infectious disease and anaemia are likely to affect the spectrum of pathology encountered. I aimed to investigate the prevalence, incidence and progression of diabetic retinopathy in Southern Malawi, to investigate the risk factors for diabetic retinopathy severity and progression in this population and to characterise endothelial function in Southern Malawian subjects with diabetes.

Methods I established the Malawi Diabetic Retinopathy Study, a 24 month prospective cohort study. Subjects were systematically sampled from two hospital-based, primary care diabetes clinics. Visual acuity, glycaemic control, systolic blood pressure, HIV status, urine albumin–creatinine ratio, and haemoglobin and serum lipid levels were assessed. Retinopathy was graded at an accredited reading centre using modified Wisconsin grading of four-field mydriatic photographs. Additionally, in order to investigate DR progression at five years, a cohort of subjects recruited to a cross-sectional study of diabetes complications in 2007 were traced and assessed. In a nested case-control study, serum markers of endothelial dysfunction and pulse amplitude tonometry were measured in a subset of subjects from the main cohort plus subjects without diabetes.

Results 357 subjects were recruited to the 24 month cohort study. At baseline 13.4% subjects were HIV-positive and 15.1% were anaemic. Baseline prevalence rates of any retinopathy, sight threatening diabetic retinopathy and proliferative retinopathy were 50.1% (95% CI 44.9–55.3), 29.4% (95% CI 24.7–34.1) and 7.3% (95% CI 4.6–10.0), respectively. Cumulative incidence at 2 years of sight threatening diabetic retinopathy for subjects with level 10 (no retinopathy), level 20

(background) and level 30 at baseline was 2.7% (95% CI 0.1-5.3), 27.3% (16.4-38.2) and 25.0% (0-67.4), respectively. In a multivariate logistic analysis, 2 step progression of diabetic retinopathy at 2 years was associated with HbA1c (odds ratio 1.27, 95%CI 1.12-1.45), baseline grade of DR (1.39, 1.02-1.91) and HIV infection (OR 0.16, 0.03-0.78). At 2 years, rates of progression to visual loss were: ≥ 15 letters lost in 17 subjects (5.8%), moderate visual impairment (< 60 letters) in 3 subjects (1.0%), severe visual impairment (< 50 letters) in 5 subjects (1.7%). The five year incidence of sight threatening diabetic retinopathy in subjects recruited to the 2007 cross sectional study, for those with level 10 and level 20 retinopathy at baseline, was 19.4% (11.3-27.4) and 81.3% (62.1-100), respectively. In the case control study of endothelial function higher serum VEGF and E-selectin were associated with having diabetes in multivariate regression. Serum VCAM-1 was associated with death in multivariate regression.

Conclusions I report the first cohort study of diabetic retinopathy from sub-Saharan Africa. I found a prevalence of sight threatening diabetic retinopathy, in subjects attending diabetes clinics, approximately 3 times that reported in recent European studies and a prevalence of proliferative retinopathy approximately 10 times higher. Progression to sight threatening diabetic retinopathy occurred approximately 3 times more frequently than reported in Europe. The negative association of HIV infection with retinopathy progression is a new finding. I report the first evidence from sub-Saharan Africa of endothelial dysfunction in subjects with diabetes and of an association between levels of endothelial biomarkers and mortality in these subjects. Results presented in this thesis highlight the urgent need for provision of services for retinopathy detection and management to avoid a large burden of vision loss.

PUBLICATIONS

Publications directly related to results presented in this thesis

- **Burgess PI**, Allain TA, García-Fiñana M, Beare NAV, Msukwa G, Harding SP. High prevalence of sight threatening retinopathy and visual impairment due to diabetes in Malawi; identification of population specific targets for intervention. *Diabetic Medicine*. 2014; 31(12): 1643-50.
- **Burgess PI**, MacCormick IJC, Harding SP, Bastawrous A, Beare NAV, Garner P. Epidemiology of diabetic retinopathy and maculopathy in Africa: a systematic review. *Diabetic Medicine*. 2013; 30(4): 399-412

Publications arising from my work in Malawi but not containing results presented in this thesis

- Bastawrous A, **Burgess PI**, Mahdi AM, Kyari F, Burton MJ, Kuper H. Posterior segment eye disease in sub-Saharan Africa: review of recent population-based studies. *Trop Med Int Health*. 2014; 19(5):600-9.
- **Burgess PI**, Msukwa G, Beare NAV. Diabetic retinopathy in Sub-Saharan Africa: meeting the challenges of an emerging epidemic. *BMC Medicine*. 2013; 11: 157
- Ellis D, **Burgess PI**, Kayange P. Management of diabetic retinopathy. *Malawi Medical Journal*. 2013; 25(4):116-20.

Manuscripts submitted or in preparation

- **Burgess PI**, Harding SP, García-Fiñana M, Beare NAV, Glover S, Cohen D, Msukwa G, Allain TA. High incidence and progression of diabetic retinopathy in Sub-Saharan Africa and relationship to HIV infection: a prospective cohort study.
- **Burgess PI**, García-Fiñana M, Harding SP, Beare NAV, Msukwa G, Kayange P, Allain TA. High mortality in subjects with both diabetes and HIV infection in Sub-Saharan Africa.

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Professor Harding created the opportunity for me to work on this project and generously provided me with support, perspective and excellent advice throughout. Without him it would not have been possible to complete this thesis. He has acted as a clinical and academic mentor to me and inspired, what I hope will be, a lifelong interest in retinal vascular disease. Professor Allain has given this project enthusiastic support from its outset. Without her many years of hard work to develop the Queen Elizabeth Central Hospital Diabetes clinic and collaboration with the World Diabetes Foundation to fund eye care in Blantyre (including a laser) this project would not have been possible. Her advice and practical support has been invaluable. Dr Garcia-Finana has brought considerable statistical expertise to this work. She has been available for guidance throughout the project and her oversight has been extremely helpful. Dr Carl Sheridan gave expert guidance on laboratory based aspects of my work for which I am very grateful.

Special thanks go to the MDRS study team. Sister Chrissy Pindani worked tirelessly throughout the project, provided practical support and vital education for our patients and expertly managed other members of the team. The excellent rapport she built with patients and their families was the cornerstone of our study's success. Our translators and research assistants Moffat Chidzuwa and Whinne Cheppe demonstrated considerable ability and professionalism. Along with Sister Pindani, they completed many days of field work in a challenging environment. The study could not have succeeded without their hard work. Ophthalmic Clinical Officers

Munthali Desire, Frank Mbewe and Owen Mkangadzula ensured the success of the study in Zomba. They showed considerable enthusiasm and pride in their work.

Dr Gerald Msukwa, Dr Petros Kayange and all the staff at the Lions Sight First Eye Hospitals in Blantyre and Zomba and the MLW Clinical Research Programme welcomed me into their institutions and were helpful and supportive despite my cultural illiteracy. I thank Dr Ticiana Criddle and Mr Stuart Lark for expertly grading retinal photographs. Despite working under pressure in a busy reading centre, they delivered high quality grading to tight deadlines. I am extremely grateful for the hard work and professionalism of Joseph Bwanali who processed, stored and catalogued laboratory samples from our patients in the MLW laboratories. I wish to thank Professor Paul Garner for his expert guidance in performing the systematic review described in the thesis. I acknowledge the generosity of the Wellcome Trust who funded both my clinical PhD fellowship and the MLW programme.

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“Give thanks to the LORD, for he is good; his love endures forever”

Psalms 118:1

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LIST OF ABBREVIATIONS

AAO	American Academy of Ophthalmology
ACCORD	Action to Control Cardiovascular Risk in Diabetes Study
ACEI	Angiotensin Converting Enzyme Inhibitors
ADA	American diabetes association
ADMA	Asymmetric dimethylarginine
ADVANCE	Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation
AGEs	Advanced glycation endproducts
AI	Augmentation index
AMD	Age-related macular degeneration
ANOVA	Analysis of variance test
ART	Anti-retroviral therapy
BCVA	Best corrected visual acuity
BIO	Binocular indirect ophthalmoscope
BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
CSMO	Clinically significant macular oedema
CRP	C-reactive protein
CWS	Cotton wool spots
dBp	Diastolic blood pressure
DCCT	Diabetes control and complications trial
DMO	Diabetic macular oedema
DPP	Diabetes Prevention Program
DR	Diabetic retinopathy
EDIC	Epidemiology of Diabetes Interventions and Complications study
ELISA	Enzyme-linked immunosorbent assay
EMPs	Endothelial microparticles
ENSP	English National Screening Programme
ETDRS	Early treatment of diabetic retinopathy study

FBS/FPG	Fasting blood sugar/Fasting plasma glucose
FFA	Fundus fluorescein angiography
FMD	Flow mediated dilation
FRHI	Framingham reactive hyperaemia index
GDP	National per capita gross domestic product
HbA1c	Glycosylated haemoglobin
HDL	High density lipoprotein
HIF1 α	Hypoxia-inducible factor 1 α
HIF2 α	Hypoxia-inducible factor 2 α
HIV	Human immunodeficiency virus
HIVAN	HIV associated nephropathy
HMa	Haemorrhages and microaneurysms
ICAM-1	Intercellular adhesion molecule 1
IDF	International diabetes federation
IMF	International monetary fund
IQR	Interquartile range
IRMA	Intra-retinal microvascular abnormalities
ICDRSS	International Clinical Diabetic Retinopathy Disease Severity Scale
LDSE	Liverpool Diabetic Eye Study
LDL	Low density lipoprotein
LOCS	Lens opacities classification system
logMAR	log of the minimum angle of resolution
LSTM	Liverpool School of Tropical Medicine
MAP	Mean arterial pressure
MDRS	Malawi diabetic retinopathy study
MLW	Malawi Liverpool Wellcome Trust Clinical Research Programme
mRCT	Meta-Register of Controlled Trials
MVL	Moderate visual loss (defined as loss of ≥ 15 letters on the ETDRS chart)
NADPH	Nicotinamide adenine dinucleotide phosphate
NCD	Non-communicable disease
NGSP	National glycohaemoglobin standardisation program

NO	Nitric oxide
NPDR	Non-proliferative diabetic retinopathy
NSC	National Screening Committee
NVD	New vessels at the disc
NVE	New vessels elsewhere
OCO	Ophthalmic clinical officer
OCT	Optical coherence tomography
OPD	Out-patient department
OR	Odds ratio
PAT	Peripheral artery tonometry
PDR	Proliferative diabetic retinopathy
PKC	Protein kinase C
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRP	Peripheral retinal photocoagulation
QECH	Queen Elizabeth Central Hospital
RCT	Randomised controlled trial
rhPAT	Reactive hyperaemia pulse amplitude tonometry index
sBP	Systolic blood pressure
SDRGS	Scottish Diabetic Retinopathy Grading System
SSA	Sub-Saharan Africa
STDR	Sight threatening diabetic retinopathy
TGF- β 1	Transforming growth factor- β 1
TNF α	Tumour necrosis factor- α
uACR	urine albumin-creatinine ratio
UKPDS	Uniked Kingdom prospective diabetes study
USD	United States Dollars
VA	Visual acuity
VCAM-1	Vascular cell adhesion molecule 1
VCT	Voluntary testing and counselling (for HIV)
VI	Visual impairment
VEGF	Vascular endothelial growth factor
WESDR	Wisconsin epidemiological study of diabetic retinopathy

WHO	World Health Organisation
ZCH	Zomba central hospital

STATEMENT OF CONTRIBUTORS TO THE MALAWI DIABETIC RETINOPATHY STUDY

I designed the Malawi Diabetic Retinopathy Study (MDRS) under the direct supervision of my PhD supervisors: Professor Simon Harding, Professor Theresa Allain, Dr Marta Garcia-Finana and Dr Carl Sheridan. Specifically Dr GarciaFinana supervised the data analysis plan and Dr Sheridan supervised design of the case-control study of endothelial function. Advice on study design was given by Mr Nick Beare, Consultant Ophthalmologist at St Paul's Eye Unit, Liverpool, Professor Alistair Craig, Professor of Molecular Parasitology at Liverpool School of Tropical Medicine (LSTM) and Dr. Gerald Msukwa, Consultant Ophthalmologist at Lions Sight First Eye Unit at Queen Elizabeth Central Hospital, Blantyre. In order to develop knowledge of techniques used to study endothelial cell biology I spent time in the laboratories of Professor Maria Grant at the University of Florida. I obtained funding for the research programme through a Wellcome Trust clinical PhD fellowship (Grant reference: 094015/Z/10/A).

All work which I completed on the MDRS was under the supervision of my PhD supervisors. I managed the research ethics committee approval process from the LSTM research ethics committee and the University of Malawi College of Medicine research ethics committee (COMREC). I designed data collection tools and, with the help of the Malawi-Liverpool-Wellcome Trust (MLW) Clinical Research Programme data team, designed, built and tested the study RedCap database. Subject recruitment and consent was performed (under my close supervision) by the MDRS study team: Research Nurse Sister Chrissy Pindani, Ophthalmic Clinical Officers Desire Munthali, Frank Mbewe and Owen Mkangadzula, Translator/Research assistants Moffat Chidzuwa and Whinnie Cheppe. After appropriate training by me, the same team collected demographic, medical history and physical examination data. Blood samples, peripheral artery tonometry measurements and point of care tests including HIV voluntary testing and counselling (VCT) were performed by Chrissy Pindani (Research Nurse). Laboratory samples were processed by MLW laboratory except for HbA1c tests which were performed at a reference lab at

Norfolk and Norwich University Hospital (see Chapter 5). I performed all enzyme-linked immunosorbent assay (ELISA) tests for markers of endothelial dysfunction at MLW laboratories.

I performed all slit lamp biomicroscopy clinical grading of diabetic retinopathy (DR). I took or directly supervised the taking of all retinal photographs to ensure adequate quality. Grading of retinal photographs for DR was performed by Ticiana Criddle and Stuart Lark (Accredited Graders at the Liverpool Reading Centre). Data entry to the study database was performed by Chrissy Pindani and myself. All data analysis detailed in this thesis was performed by me. I performed all laser treatment and follow-up clinics for research subjects during the course of the study. Clinical work was supervised by Dr Gerald Msukwa, Mr Nicholas Beare and Professor Simon Harding. As part of the MDRS a cohort of subjects originally seen in 2007 was traced and re-examined. In the original 2007 study clinical grading of DR was performed by Mr. Simon Glover. Demographic and medical assessments were performed by the QECH diabetes clinic team as detailed in the original publications [1,2].

Introduction to Thesis

Diabetes has been recognised in Africa for over 100 years. In 1901 Albert Cook, a medical missionary in Uganda, reported that “diabetes is rather uncommon and very fatal” [3]. However, it was not until the 1960s that the first reports on diabetic retinopathy (DR) appeared in the medical literature [4,5,6]. Present day Sub-Saharan Africa (SSA) is in the midst of a health transition characterised by coexisting epidemic infectious disease and a rise in non-communicable disease (NCD), in societies facing high levels of perinatal and maternal disorders, child mortality and trauma. The International Diabetes Federation has estimated that the number of adults with diabetes in Africa will increase from 12.1 million in 2010 to 23.9 million in 2030 [7] a presumed consequence of poor diet, sedentary lifestyles, obesity, and population growth and ageing (in part due to successes in combating communicable diseases). Diabetes results in considerable morbidity, disability and early mortality. The epidemic rise in diabetes poses significant public health and socioeconomic challenges for the continent.

Diabetes causes visual impairment through early onset cataract and DR, a progressive disease of the retinal microvasculature. Cataract and DR are the second and sixth leading causes of global visual impairment, respectively [8]. Both are included in the list of nine target diseases of the 'Vision 2020 Action Plan' a joint program of the WHO and the International Agency for the Prevention of Blindness. The prevalence and incidence of sight threatening diabetic retinopathy (STDR) in developed countries have been well documented [9,10,11]. Associations between systemic factors, including glycaemic control [12,13], blood pressure [14] and blood lipid levels [15] and the development and progression of retinopathy in these populations are well known. In contrast there is a paucity of evidence on the epidemiology of DR in Africa. In this resource-poor setting population-specific variables such as a high burden of infectious disease (including HIV and malaria), and anaemia are likely to affect the spectrum of pathology encountered.

Malawi (population 15.9 million) is one of the poorest countries in Southern Africa, with an annual per capita healthcare expenditure of US\$77 [16]. The 2009 WHO Malawi National STEPwise Survey estimated a prevalence of diabetes of 5.6% in adults 25-64 years, with similar prevalence in rural and urban areas [17]. In 2007 a survey of diabetes complications was performed in patients attending the diabetes clinic at Queen Elizabeth Central Hospital (QECH), Blantyre [1]. This study reported a high prevalence of STDR and proliferative retinopathy (PDR) in subjects examined by slit lamp biomicroscopy: 19.6% and 5.7%, respectively [2].

In response to these important findings I set out to elucidate the determinants of severity and progression of DR in Southern Malawi. This thesis, completed as part of a Wellcome Trust Clinical PhD Fellowship, outlines a program of both clinical and laboratory based research addressing the clinical determinants of DR severity and progression as well as the underlying cellular mechanisms. The objectives of the thesis were the following:

1. To investigate the prevalence, incidence and progression of DR in Southern Malawi
2. To investigate the risk factors for DR severity and progression in this population
3. To characterise endothelial function in Southern Malawian subjects with diabetes and to investigate relationships with severity of retinopathy

This work provides valuable baseline epidemiological data for DR in Sub-Saharan Africa, delivers novel insights into the pathophysiology of this vascular disease and will guide future intervention studies in the region.

Chapter 1. Pathophysiology of Diabetic Retinopathy

1.1 Aims of the chapter

The first 4 chapters of this thesis comprise my literature review. In chapters 1, 2 and 3 I review the current literature concerning the pathophysiology of diabetic retinopathy (DR), grading of DR and determinants of severity and progression of retinopathy, respectively. In Chapter 4 I report a systematic review of the epidemiology of diabetic retinopathy and maculopathy in Africa, summarise the findings of the literature review and present the hypothesis and aims of my thesis. In this first chapter I describe the normal anatomy and physiology of the retina and present an overview of the pathophysiological processes involved in DR. Particular focus is given to the role of the vascular endothelium.

1.2 Overview

Diabetes has many manifestations in the eye. The most significant causes of visual impairment are cataract and DR. A variety of pathological processes are thought to contribute to the development of DR including dysfunction of the vascular endothelium, chronic low grade inflammation and changes in leukocyte cell biology. Progressive damage to the retinal microvasculature leads to the clinical manifestations of the disease which, in its early stages, is asymptomatic. The pathophysiology of DR is a highly complex topic with an extensive literature. The literature review presented in this chapter will examine in depth only those areas relevant to this thesis.

DR can be broadly divided into clinical categories: background DR and pre-proliferative DR (collectively described in North American literature as non-proliferative DR (NPDR)) and proliferative (PDR). NPDR is characterised by abnormal permeability and/or non-perfusion of capillaries leading to retinal ischaemia. Diabetic maculopathy occurs when these processes affect the macula and are therefore a threat to visual functioning. Clinically significant macular

oedema (CSMO) is a term from the Early Treatment of Diabetic Retinopathy Study (ETDRS) [19] and is an evidence based threshold for laser photocoagulation treatment. PDR occurs when retinal ischaemia is sufficiently severe to lead to the formation of new vessels. Visual loss occurs in PDR when these vessels bleed, or tractional retinal detachment ensues from fibrovascular proliferation. Without treatment, 50% of patients with PDR will become blind within 5 years [18]. Laser photocoagulation has been shown to be effective at reducing the likelihood of visual impairment and blindness in patients with PDR [18] and macular edema [19] if timely treatment is performed.

1.3 Retinal anatomy

Like the rest of the central nervous system, the retina is embryologically derived from the neural tube. The retina is the innermost of the three principle layers of the eye (the others being the corneo-scleral tract and the uveal tract) (Figure 1.1). The retina consists of 2 primary layers: an inner neurosensory retina and an outer retinal pigment epithelium. The sub-retinal space is a potential space between these two layers. The neurosensory retina is only attached firmly at the optic disc and anteriorly at the ora serrata. While a number of definitions for the macula exist, that used by the Fundus Photograph Reading Center at the University of Wisconsin is a circular zone of retina with radius 3.8mm centred on the foveal centre (Figure 1.2). This region is dominated by cone photoreceptors and histologically is seen to have more than a single layer of ganglion cell bodies. The fovea is a 1.5mm diameter area at the centre of the macula, the centre of which is 4.5mm temporal to the centre of the optic disc. The centre of the fovea is a depression surrounded by thickened margins where cone photoreceptors are concentrated at maximum density.

The retina is one of the most metabolically active tissues in the body. In humans the retina has a dual blood supply: the inner two thirds are supplied by branches of the central retinal vessels, the outer one third is supplied by the choroidal circulation (both circulations are branches of the ophthalmic artery) [20]. An area at the centre of the fovea contains no retinal capillaries: the normal diameter of the

foveal avascular zone is less than $600\mu\text{m}$. Ischaemic maculopathy can be diagnosed when the avascular zone is over $1000\mu\text{m}$ in diameter; between 600 and $1000\mu\text{m}$ is intermediate between the two states.

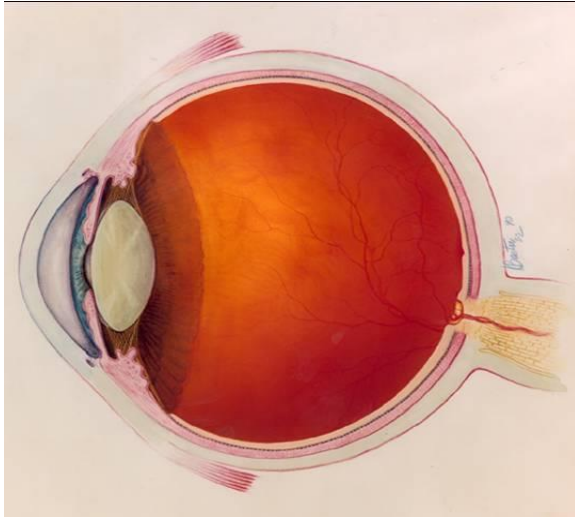


Figure 1.1 Cross section of the human eye demonstrating the 3 principle layers of the globe: (1) corneo-scleral tract (white), (2) uveal tract comprising choroid, ciliary body and iris (pink), and (3) retina (red). Reprinted with permission [20].

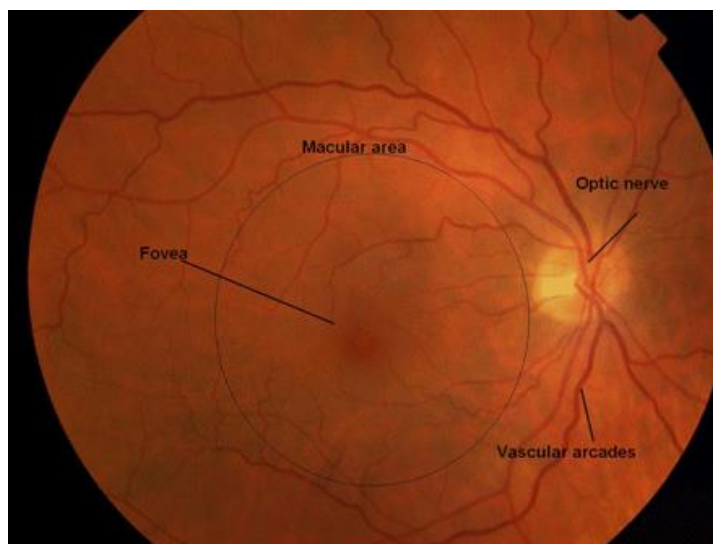


Figure 1.2 Normal retinal anatomy of the human eye. Position of the macula, fovea, optic nerve head and the vascular arcades marked. (Courtesy of Liverpool Diabetic Eye Study).

1.4 Retinal physiology

The retinal changes associated with diabetes have been described by Antonetti, Klein and Gardner [21] as dysfunction of the retinal neurovascular unit. Neuronal cells, glia and pericytes and specialised vasculature make up this neurovascular unit (Figure 1.3). Close physical and biochemical relationships between these cells contribute to important functions of the retina. The blood-retinal barrier controls flow of fluids, metabolites and nutrients between the blood and the neural retina. The inner blood retinal barrier comprises non-fenestrated endothelial cells of the retinal capillaries and the tight junctions between these cells. The outer blood retinal barrier is made up of retinal pigment epithelial cells and their intercellular tight junctions [20]. Interaction of glia and neurones are required for neurotransmitter release, energy balance and to maintain the proper ionic environment for neuronal signalling.

In the ganglion cell layer the cell bodies of ganglion cells and astrocytes are in close proximity to retinal capillaries which provide their nutrients and oxygen. A further capillary bed in the inner nuclear layer nourishes the amacrine and Muller cells. Retinal vessels do not receive an autonomic nerve supply. Auto-regulation of retinal blood flow is mediated by pericytes and by levels of metabolites including lactate and carbon dioxide [22]. The outer retina receives oxygen and nutrients from the choroidal circulation. Muller cells and photoreceptors are metabolically coupled to facilitate the production of electrochemical impulses following stimulation with light. The retinal pigment epithelium provides support to the highly metabolically active photoreceptors.

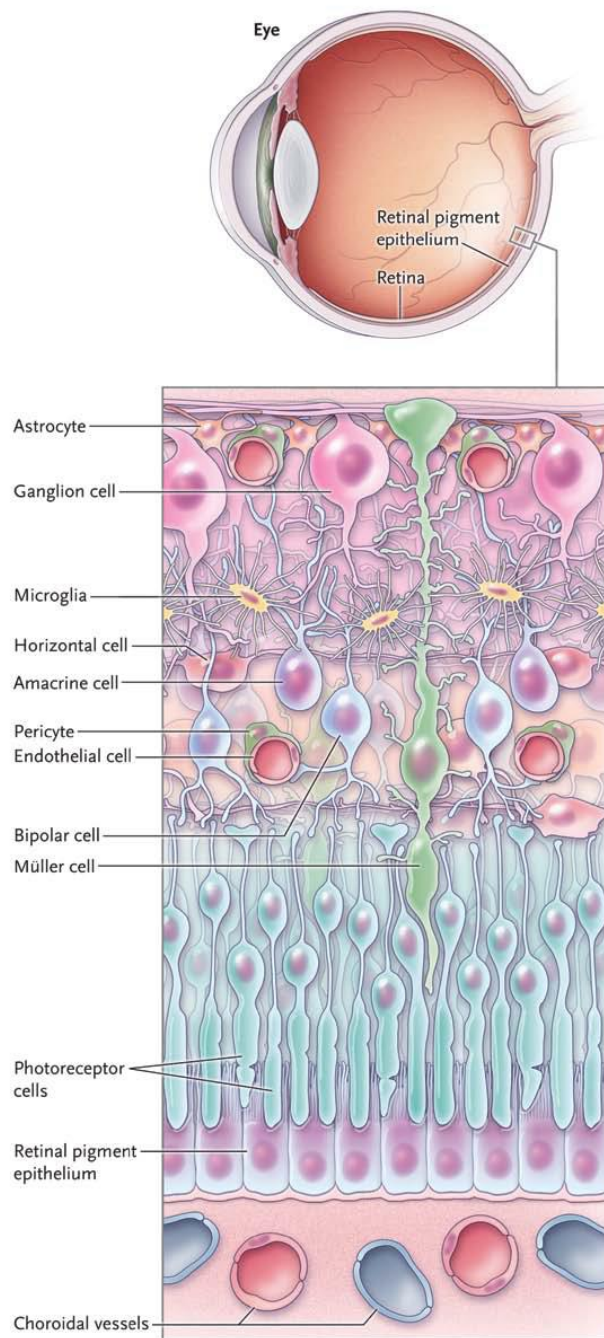


Figure 1.3 The retinal neurovascular unit. Vascular endothelial cells, pericytes, astrocytes and Muller cells in close proximity supporting the function of the ganglion cells and neural retina.

1.5 Pathophysiology of DR

Here follows a brief overview of the pathophysiology of DR to provide background to the thesis.

1.5.1 Histopathological lesions

A number of anatomical lesions are well described in DR. Pericytes are contractile cells which are important contributors to autoregulation of the microvasculature. Loss of these cells is an early and specific sign of DR. Clinically pericyte loss and loss of vessel tone manifests as 'venous beading' and venous dilation. Loss of intercellular contacts may promote endothelial cell proliferation and microaneurysm formation [23]. A number of changes in the capillary basement membrane are reported from electron microscopy in DR. Thickening, deposition of fibrillar collagen and formation of vacuoles are associated with glycation of basement membrane collagen by enzymatic and non-enzymatic processes [24]. These changes are thought to be critical in loss of inter-cellular signalling and therefore normal function of pericytes and endothelial cells.

Microaneusyms (MA) are not specific to DR but are the earliest clinical sign of the disease (Figure 1.4). MA are located in the inner retina and either acellular or hypercellular. On ophthalmoscopy they appear as small, intraretinal red dots; on fluorescein angiography dots of hyperfluorescence show variable leakage in later images. The mechanism of MA formation remains to be elucidated. Pericyte loss may contribute by weakening the capillary wall and loss of anti-proliferative effect [23]. However, MAs occur in other diseases of the retinal microvasculature in which pericyte loss is not observed [25].

Loss of the cellular elements of retinal capillaries is accompanied by endothelial dysfunction, leucocyte adhesion and changes in retinal blood flow [26]. 'Capillary dropout' describes the appearance on fluorescein angiography of non-perfused areas of the retinal capillary bed. Again this finding is not specific to DR and the mechanisms of capillary cell death are poorly understood. Breakdown of the blood

retinal barrier results in vision threatening macular oedema. A key mechanism is loss of tight junctions between vascular endothelial cell processes [27]. An important mediator of blood retinal barrier breakdown, vascular endothelial growth factor (VEGF) alters endothelial cell tight junctions. Components of the kallikrein–kinin system including plasma kallikrein, factor XII, and kininogen are also involved in retinal vascular permeability through effects on bradykinin [23].

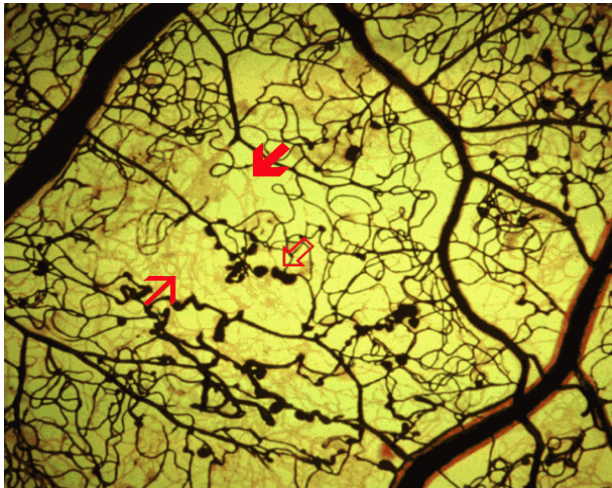


Figure 1.4 Trypsin digest preparation of human retina showing pathological changes of diabetic retinopathy. Arrows indicate loss of retinal capillaries (thick solid arrow), capillary non-perfusion (thin solid arrow), and capillary microaneurysms (arrow outline). Reprinted with permission [21].

1.5.2 Biochemical mechanisms

Long term hyperglycaemia is the initial factor leading to the development of DR. The mechanisms by which hyperglycaemia leads to the development of DR are not fully understood. A number of theories are proposed which are not mutually exclusive.

The aldose reductase theory The aldose reductase (polyol or sorbitol) pathway is a series of intracellular reactions involving sorbitol dehydrogenase and aldose reductase. The latter enzyme reduces aldose sugars into their respective sugar alcohols. Glucose is reduced to sorbitol which is then oxidised into fructose by sorbitol dehydrogenase. Hyperglycaemia leads to the activation of the aldose

reductase pathway. The sorbitol dehydrogenase reaction is slower than the aldose reductase reaction. The cell membrane is poorly permeable to sorbitol therefore high intracellular levels accumulate. Osmotic stress may account for some of the pathological changes seen in DR. The aldose reductase pathway utilises the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). Activation of this pathway in hyperglycaemia may reduce intracellular NADPH, alter intracellular redox balance and result in oxidative stress [28]. Interestingly, aldose reductase inhibitors have been effective in reducing signs of DR in animal models but not human trials [29].

Advanced glycation endproduct theory In the presence of hyperglycaemia proteins, lipids and nucleic acids may undergo modification by sugar derived products to form advanced glycation endproducts (AGEs). Formation of AGEs may impair function of intra and extra cellular proteins and this has been proposed as a mechanism to explain vascular damage in diabetes [30]. AGEs bind to receptors including the immunoglobulin superfamily member 'Receptor for AGE' and the macrophage scavenger receptor. Activation of intracellular kinases may lead to cellular dysfunction [31]. Inhibitors of the AGE pathway have been successful in preventing many effects of hyperglycaemia in animal studies [32] but human trials have been limited by toxicity of these agents.

Reactive oxygen intermediates theory A further theory of the pathophysiological mechanisms in DR focusses on reactive oxygen intermediates. There is evidence of oxidative stress in patients with diabetes: lower levels of the antioxidants vitamin C, vitamin E and glutathione [33] and raised levels of markers of oxidative stress including oxidised low density lipoprotein (LDL) cholesterol [34] are reported. Glucose is metabolised through glycolysis and oxidative phosphorylation in the mitochondria. Free radicals are by-products of this reaction. It is proposed that hyperglycaemia results in increased free radical production and consequent oxidative stress [23]. In animal models antioxidants have reduced development of the microvascular complications of diabetes [35] but trials in humans have not shown encouraging results.

Protein Kinase C theory Hyperglycaemia (as well as reactive oxygen species, AGEs and VEGF) results in pathological activation of protein kinase C (PKC). The actions of this enzyme lead to increased vascular permeability, increased leucocyte adhesion to vascular endothelium, dysregulation of nitric oxide synthesis and alteration in retinal blood flow [36]. The PKC inhibitor ruboxistaurin is reported to prevent early vascular changes in retinopathy, neuropathy and nephropathy [37]. The prospective Protein Kinase C Diabetic Retinopathy Study did not show a difference in progression to sight threatening maculopathy or focal/grid laser at 30 months [38]. However, an open label extension showed a reduction in moderate visual loss (MVL) in those subjects with most exposure to ruboxistaurin [39]. Some commentators have suggested that use of a higher dose of ruboxistaurin in the initial trial may have resulted in a significant difference in favour of treatment.

1.5.3 Inflammation in DR

Systemic inflammation occurs in response to obesity and diabetes. Circulating monocytes from subjects with diabetes, compared with controls, show greater production of superoxide and pro-inflammatory cytokines including interleukin-1, interleukin-6, and tumour necrosis factor- α (TNF α) [40]. Transforming growth factor- β 1 (TGF- β 1), a pleiotropic factor with predominantly immunosuppressive effects, is elevated in the serum of persons with diabetes [41]. Extensive evidence in humans and animal models supports the role in DR of persistent low-grade inflammation involving an influx of inflammatory effectors, both cytokines and leukocytes.

Retinal inflammatory mediators including VEGF, interleukin-1 β , TNF α , intercellular adhesion molecule-1 (ICAM-1) and angiotensin II are up regulated in diabetes [42]. Activation of microglial cells occurs in early DR [43]. Activation of leucocytes and leucostasis has been demonstrated in animal models; the latter process dependent on ICAM-1 and its ligand CD18 [44]. Leucocyte activation and adhesion appears to play an important role in vascular endothelium dysfunction. Exposure to chronic infections such as HIV or repeated episodes of acute infection (e.g. malaria) may

modify the systemic and local inflammatory response to hyperglycaemia. The effects of infection on DR and the cellular mechanisms underlying these relationships are a theme in this thesis.

1.5.4 Dysfunction of the vascular endothelium

The endothelium plays a pivotal role in vascular health. Adherence of leukocytes is associated with direct injury to and apoptosis of endothelial cells and with disruption of endothelial tight junctions [45]. Both diabetes and its complications are associated with altered serum levels of biomarkers of endothelial dysfunction. Markers of endothelial dysfunction including soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and von Willebrand factor (vWf), are associated with macroangiopathy in subjects without diabetes and those with type 2 diabetes [46,47]. Serum levels of sICAM-1, sVCAM-1 and CRP are reported to be elevated in subjects with DR [48, 49]. Elevated E-selectin in patients with type 2 diabetes is associated with presence of DR [49] and progression of DR [50]. VEGF is a mitogen for endothelial cells, and its expression both in vivo and in vitro can be induced by hypoxia [51]. Serum VEGF is elevated in diabetic compared to control subjects [52,53,54]. A number of studies have demonstrated elevated VEGF in diabetic subjects with DR when compared to those without retinopathy [52,55]. The interaction of DR with other pathologies which induce dysfunction of the vascular endothelium is poorly understood. HIV infection and many of the antiretroviral agents used to treat the condition are associated with changes in leukocyte cell biology, vascular endothelium dysfunction and vascular complications including retinopathy [56].

1.5.5 Assessment of the vascular endothelium

Serum biomarkers of endothelial dysfunction Assessment of vascular endothelial function is challenging due to its location. Cytokines such as TNF α and interleukin-1 are pleiotropic proteins which regulate activity of leucocytes. They participate in the cascade leading to greater expression of the leucocyte and vascular endothelial adhesion molecules including ICAM-1 and VCAM-1. Serum levels of both cytokines

and adhesion molecules are elevated in diseases associated with vascular endothelial dysfunction such as diabetes (described above in Section 1.8). Endothelial microparticles (EMPs) are small vesicles that are released into the circulation from damaged or activated vascular endothelial cells [57]. EMPs released by apoptotic and activated cells exhibit different characteristics. Serum levels correlate with clinical findings in coronary artery disease [58] and renal failure [59]. Serum biomarkers are accessible and measurement requires only simple enzyme-linked immunosorbent assay (ELISA) techniques. However, serum levels reflect global endothelial dysfunction; specific areas of the vascular bed, which may exhibit particular functional characteristics, are not measured individually. Serum markers do not directly measure specific functional properties of the vascular endothelium. The establishment of the validity of particular biomarkers was beyond the scope of this thesis.

Measurement of *in vivo* endothelial function The current reference standard for assessing endothelial function is coronary angiography with infusion of the endothelium-dependent vasodilator acetylcholine. Vasodilation occurs due to receptor-mediated release of nitric oxide (NO) [60]. Clear disadvantages of this test are its highly invasive nature and the need for highly skilled interventional radiologists. A much less invasive method is measurement of 'flow mediated dilation' (FMD) of the brachial artery. The artery is occluded by external pressure with a sphygmomanometer cuff; release causes reactive hyperaemia and shear stress. Artery diameter is measured before and after occlusion by high-resolution ultrasound. The degree of dilation chiefly reflects endothelial function dependent on bioavailability of NO [61]. The principle disadvantage of this method is that it requires a highly skilled operator.

A number of non-invasive, operator-independent tests have the capacity to measure endothelial function. Peripheral artery tonometry (PAT) measures digital pulsatile volume changes during reactive hyperaemia following upper arm blood flow occlusion. The expected response is of a post occlusion increase of the PAT signal amplitude. This response is at least 50% dependent on endothelial NO

activity [62]. The test has been validated against the FMD method [63] and has been used in the third-generation Framingham Heart Study cohort [64]. Decreased PAT response has been demonstrated in subjects with diabetes and others risk factors for vascular disease [65,66]. It is not currently possible to measure retinal vascular endothelial function *in vivo*. Measurement of retinal blood flow is achievable but is influenced by numerous factors besides function of the endothelium [67].

1.6 Clinical manifestations of DR

1.6.1 Natural course of DR

DR is a microangiopathy chiefly affecting the pre-capillary arterioles, capillaries and post-capillary venules. Five pathological processes underlie the natural course of DR: formation of capillary MA; increased vascular permeability; eventual vascular non-perfusion; proliferation of new blood vessels (which may bleed) and fibrovascular tissue; and contraction of fibrovascular tissue and the vitreous leading to tractional retinal detachment. The disease can be classified into 3 stages on the basis of clinical features: (1) 'background' in which pathological changes are intraretinal; (2) 'pre-proliferative' the features of which precede proliferation of new vessels and fibrovascular tissue; and (3) proliferative in which the pathology extends onto or out-with the retina. Diabetic maculopathy describes changes of DR beyond the background stage affecting the macula.

1.6.2 Background diabetic retinopathy

Microaneurysms Retinal capillary MAs are typically the earliest sign of DR which can be detected on clinical examination. Histopathological characteristics of MAs are described above in Section 1.5. Proposed mechanisms for MA formation include aborted vasoproliferation, weakness of the capillary wall secondary to pericyte loss, and increased intraluminal pressure [68]. MAs are located in the inner nuclear layer of the retina and range in diameter from 15 to 100 μm . The walls of early MAs are

transparent; they appear as small red dots the same colour as retinal veins. The natural history of MAs sees thickening of the wall with eventual luminal occlusion, a process which takes approximately 18 months but is influenced by treatment of diabetes [69]. As the wall thickens the colour of a MA changes from red to orange and finally to white.

Haemorrhages Intraretinal haemorrhages appear as 'dots' and are located in the middle layers of the retina. These haemorrhages arise from the venous end of capillaries. Dot haemorrhages can be very difficult to distinguish from MAs on clinical examination. Recent evidence from studies using optical coherence tomography (OCT) angiography suggests that there are other vascular abnormalities that may appear similar to MA including capillary loops and telangiectasia [72]. Haemorrhages from pre-capillary arterioles occur in the retinal nerve fibre layer and are described as 'flame shaped' (Figure 1.5). Retinal nerve fibre layer haemorrhages also occur in the absence of diabetes and are associated with hypertension. Other early features of DR include capillary dilation and venous dilation which may be present before MAs and occurs in up to 10% of subjects [68].

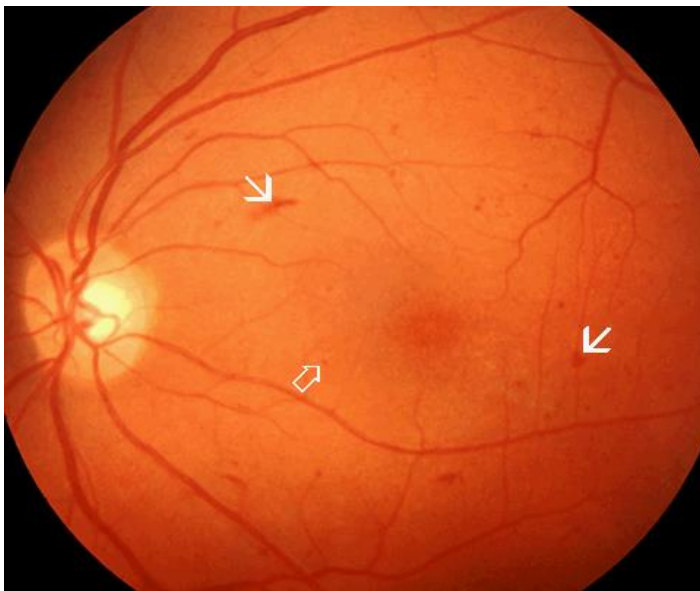


Figure 1.5 Colour fundus photograph demonstrating background diabetic retinopathy. Arrows indicate flame shaped haemorrhage (superior temporal to optic disc), microaneurysm (temporal to optic disc) and blot haemorrhage (temporal to the fovea). (Courtesy of Liverpool Diabetic Eye Study).

1.6.3 Preproliferative diabetic retinopathy

Progression of DR is seen on fluorescein angiography as hypofluorescent areas of capillary non-perfusion. Angiographic features are accompanied by the clinical features of preproliferative diabetic retinopathy (PPDR): deep round 'blot' haemorrhages, 'cotton wool spots', intra-retinal microvascular abnormalities (IRMA) and venous changes. Presence of these feature correlates with risk of development of PDR [70] (this evidence is reviewed in Chapter 2 of this thesis).

Blot haemorrhages In contrast to dot haemorrhages, blot haemorrhages represent deep retinal infarcts located in the outer plexiform layer of the retina. They are found where clusters of capillaries occlude.

Cotton wool spots (CWS) CWS are white lesions with indistinct margins located in the superficial retinal layers (Figure 1.6). They comprise localised accumulations of axoplasmic debris with adjacent bundles of unmyelinated ganglion cell axons. Traditionally CWS were thought to represent focal infarcts from terminal arteriolar occlusion in the retinal nerve fibre layer. However, an alternative hypothesis of CWS as boundary sentinels of inner retinal ischaemia has been suggested [71].

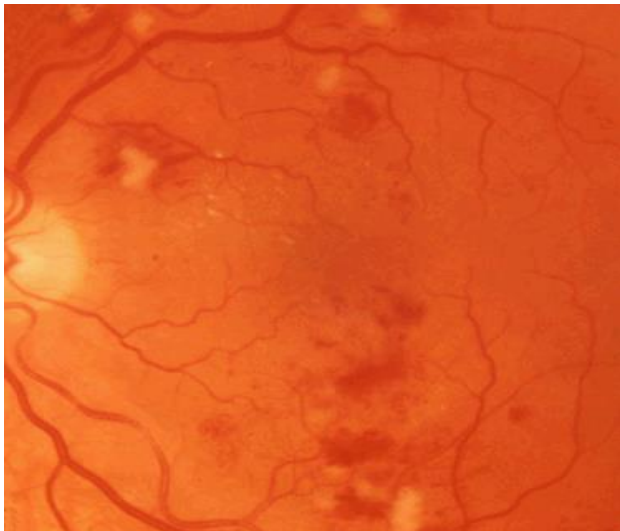


Figure 1.6 Colour fundus photograph demonstrating multiple white 'cotton wool spots' and large dark 'blot' haemorrhages along the vascular arcades. (Courtesy of Liverpool Diabetic Eye Study).

Intraretinal microvascular abnormalities (IRMA) IRMA are vascular shunts joining retinal pre-capillary arterioles to venules. On clinical examination they appear as intraretinal fine red lines which may have a tortuous configuration (Figure 1.7). Angiography demonstrates that these lesions are usually located adjacent to areas of capillary non-perfusion. IRMA may be difficult to differentiate from new vessels. In contrast to neovascularisation they show no, or only mild, leakage on fluorescence angiography, do not cross major retinal vessels and are wholly intraretinal.

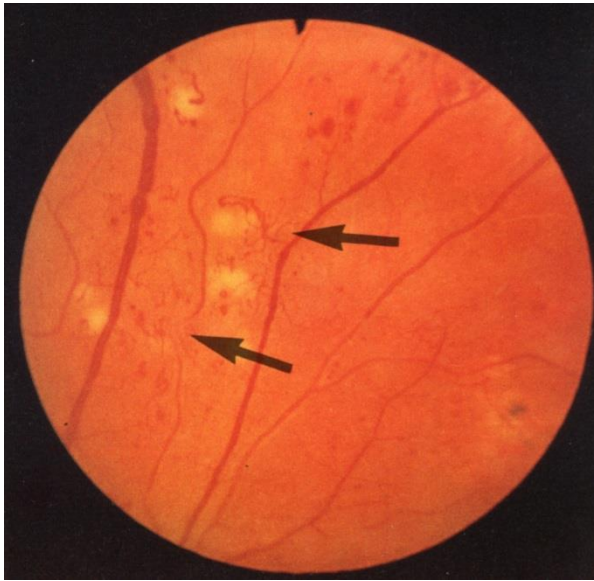


Figure 1.7 Colour fundus photograph demonstrating intraretinal microvascular abnormalities (IRMA)(arrows). The large calibre vein on the left of the photograph demonstrates 'beading' (Courtesy of Liverpool Diabetic Eye Study).

Venous changes Where veins traverse areas of extensive capillary closure venous changes occur. The most common change is venous beading. Beading may occur due to loss of supporting tissue architecture or may represent foci of venous endothelial cell proliferation which failed to develop into neovascularisation. Infrequent abnormalities include venous loops and venous reduplication.

1.6.4 Proliferative diabetic retinopathy

PDR is the vascular response of the retina to extensive capillary closure. Tissue ischaemia is thought to lead to production of angiogenic factors including hypoxia-

inducible factor 1 α (HIF1 α), HIF2 α and VEGF [68]. The clinical features of PDR are new vessels at the optic disc (NVD), new vessels elsewhere (NVE), pre-retinal/vitreous haemorrhage and proliferation of fibrous tissue.

New vessels at the disc (NVD) New vessels arise most commonly at the posterior portion of the fundus: usually within 45 degrees of the optic disc. NVD are defined as neovascularisation arising on the disc or within 1 disc diameter of it (Figure 1.8). The normal disc has a network of fine capillaries on the surface: the pre-papillary capillary plexus. New vessels are distinguished from normal vessels by looping back to the disc, forming loops in which the top of the loop is wider than the base, presence of solid tips and variable vessel calibre. If there is doubt NVD demonstrate profuse leakage on fluorescein angiography.

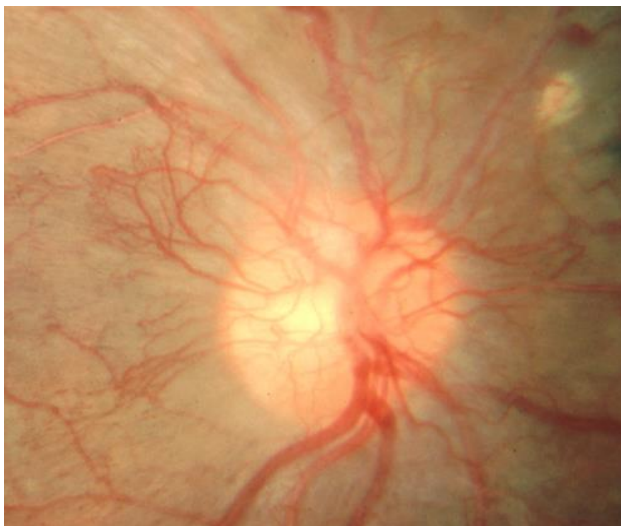


Figure 1.8 Colour photograph showing new vessels at the disc (NVD)(Courtesy of Liverpool Diabetic Eye Study).

New vessels elsewhere (NVE) New vessels arise from post-capillary venules and occur at the watershed between perfused and non-perfused retina. Unlike normal retinal vessels they do not obey the law of fractals (Figure 1.9). In the presence of more advanced ischaemia new vessels may form on the iris; neovascularisation within the anterior chamber angle forms a barrier to aqueous drainage and leads to neovascular glaucoma.

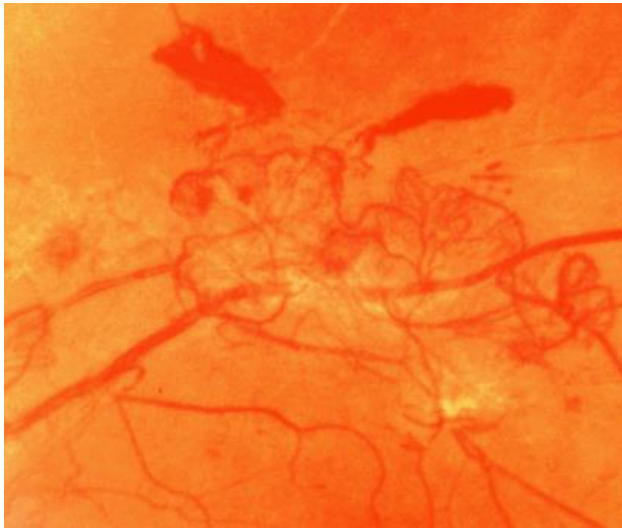


Figure 1.9 Colour photograph showing a large area of new vessels elsewhere (NVE). (Courtesy of Liverpool Diabetic Eye Study).

Fibrous tissue New vessels undergo proliferation and regression in a dynamic cycle [68]. Initially non-fibrous they may develop fibrous tissue. This tissue is at first translucent but becomes progressively opaque. Dynamic interaction at the vitreoretinal interface results in an inflammatory response, increased scar formation and adhesions between the retina and vitreous, in a “wound healing” response. New vessels and fibrous tissue are asymptomatic until vitreoretinal interaction leads to pre-retinal or vitreous haemorrhage or contraction of the vitreous precipitates tractional retinal detachment.

1.6.5 Advanced diabetic retinopathy

Pre-retinal and vitreous haemorrhage Proliferating vessels grow into the vitreous gel. These new vessels are fragile; traction from the gel results in sub-hyaloid/pre-retinal haemorrhage (Figure 1.10). When blood breaks the posterior hyaloid surface it is described as vitreous haemorrhage.

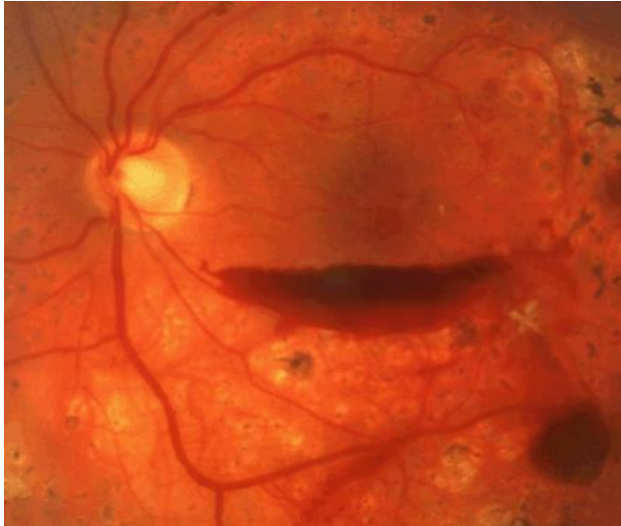


Figure 1.10 Colour photograph showing a typical 'boat shaped' pre-retinal haemorrhage: blood is held behind the posterior hyaloid face and assumes this shape due to gravity. Lesions of peripheral scatter laser are present. (Courtesy of Liverpool Diabetic Eye Study).

Tractional retinal detachment Contraction of fibrovascular tissue and the adherent vitreous gel results in traction on the retina. When tractional forces overcome adhesion between the retina and the underlying retinal pigment epithelium retinal detachment occurs. This process progresses slowly unless retinal breaks occur leading to a combined tractional and rhegmatogenous (due to a retinal break) detachment (Figure 1.11).

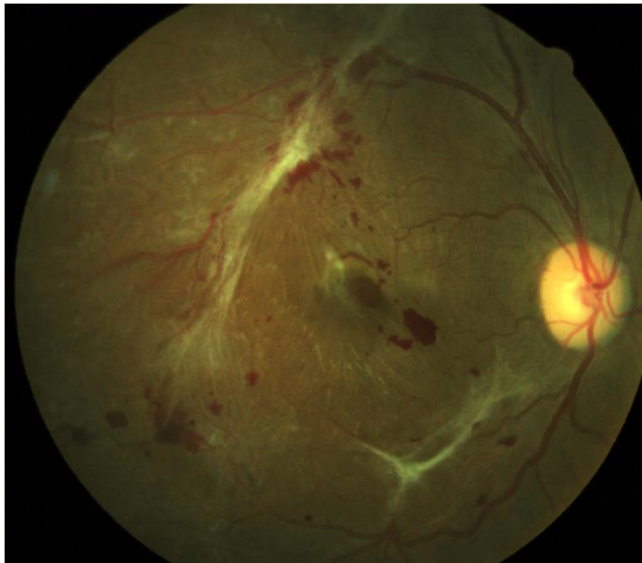


Figure 1.11 Colour photograph showing extensive, elevated fibrovascular tissue on either side of the fovea. Lines in the retinal tissue indicate traction across the fovea. The perifoveal, dark, ovoid lesion is a retinal hole (MDRS photographs).

1.6.6 Diabetic maculopathy

Macular oedema Thickening of the retina occurs via two mechanisms. Abnormal capillary permeability (breakdown of the blood retinal barrier) leads to passage of fluid from the vascular compartment to surrounding retinal tissue. Hypoxia leads to accumulation of intracellular fluid in retinal cells. Leakage from clusters of MAs may appear as a discrete area of ‘focal’ oedema (Figure 1.12). More widespread ‘diffuse’ oedema is thought to result from leakage from capillary segments as well as MAs. Retinal pigment epithelial dysfunction may also contribute [68]. Macular oedema disrupts the normal architecture of the retina and threatens vision.

Exudates Retinal thickening can only be appreciated on stereoscopic viewing or OCT. A surrogate marker of thickening is the presence of exudates. These yellow, waxy and sharply demarcated lesions are composed predominantly of lipids and located in the outer plexiform layer. When found in a circular or semi-circular configuration surrounding an area of thickening they are described as ‘circinate exudates’ (Figure 1.13). Lipid remains dispersed within the retina in the area of

oedema but is deposited at the edges as water and small molecules are reabsorbed by surrounding non-ischaemic tissue [68].



Figure 1.12 Left: Fluorescein angiogram images from a subject with diabetic macular oedema: leaking microaneurysms close to the centre of fovea (yellow arrow); foveal avascular zone (blue arrow). Right: Optical coherence tomography scan showing a cross section through the retina: intraretinal (black) fluid cysts indicate the presence of diabetic macular oedema.



Figure 1.13 Colour photograph demonstrating a 'circinate ring' of exudates surrounding a cluster of microaneurysms. In this example the exudates are not close to the central fovea; vision was Snellen 6/5. (Courtesy of Liverpool Diabetic Eye Study).

Ischaemia Capillary non-perfusion at the macula leads to ischaemia and dysfunction of the surrounding retinal tissue. At present no specific treatments are available for ischaemic maculopathy. However, medical management of diabetes and fractionated scatter laser treatment are thought to be beneficial. Clinical clues to the diagnosis include poor vision (6/36 Snellen or worse), macular IRMA, blot haemorrhages and vessel 'pruning'. Definitive diagnosis is by fluorescein angiography. Ischaemia may occur alone or in combination with leakage.

1.7 Chapter summary

The pathophysiology of DR is complex. Dysfunction of the vascular endothelium is an important component and can be assessed by measurement of serum biomarkers and *in vivo* functional testing. The clinical features of DR are well described but with the introduction of OCT and antiVEGF therapy are changing. In Chapter 2 I will detail the various existing DR grading systems and review the evidence relating grades of retinopathy with risk of progression of retinopathy and risk of visual loss.

Chapter 2. Grading of Diabetic Retinopathy

2.1 Aims of the chapter

There are many methods of classifying diabetic retinopathy (DR). In this chapter I describe the benefits and drawbacks of the existing systems and introduce the Liverpool Diabetic Eye Study (LDES) grading scheme.

2.2 Overview

In Chapter 1 of this thesis I detailed the clinical features of DR. Classification is primarily based on the presence of these features (feature specific grading), examined either by ophthalmoscopy or using photography. An ideal grading system would be sensitive, specific, reproducible by clinicians, technicians, graders and researchers, validated for prognosis in large cohort studies and quick to perform. Existing systems represent a compromise between these qualities.

2.3 Historical grading systems

The Hammersmith Hospital Grading System The forerunner of current feature specific grading systems based on comparison with standard photographs, this system was originally published in 1967 by Oakley and colleagues [73]. It was designed for assessment of patients undergoing pituitary ablation for DR. Each of four features: microaneurysms, haemorrhages, exudates and fibrous proliferations, were graded on a five point scale against standard photographs. In 1972 this system was modified by Kohner *et al.* [74] to include classification of new vessels and cotton wool spots (CWS). This classification was not validated for prognosis of visual outcome.

The Oxford Retinopathy Index This system for classifying early DR involves counting individual microaneurysms from colour fundus photographs at 25 fold

magnification. It was originally published by Howard-Williams in 1986 [75]. Only a small area of the retina is examined. This system has not been validated against other grading systems but is a sensitive indicator of DR progression [76].

2.4 Airlie House classification

In 1968 a group of experts met at Airlie House, Virginia. An important outcome of this symposium was the development of a standardised classification of DR. The Airlie House system classified DR into background and proliferative. Proliferative DR (PDR) was subdivided according to the presence of new vessels, fibrous proliferations and vitreous haemorrhage. Each feature was graded according to location and extent [77]. This system was subsequently modified and used in the Diabetic Retinopathy Study (DRS) [78]. This randomised, controlled clinical trial commenced in 1971 and involved 1,758 subjects. The DRS assessed the effectiveness of Xenon and Argon laser treatment for treating PDR. At 2 years, photocoagulation significantly reduced the risk of severe visual loss (defined as visual acuity 5/200 or worse; approximately equivalent to Snellen 2/60) by approximately 50% compared to no treatment [79]. The benefit persisted throughout 5 years of follow-up [80]. The benefits of laser treatment were greatest in patients with features of DR termed 'high risk characteristics' by the DRS (Box 2.1) [81]. The Airlie House classification system was useful for making comparisons between different treatment modalities. However, it was insensitive to changes in early features of retinopathy.

Box 2.1 High risk characteristics for severe visual loss defined by the Diabetic Retinopathy Study [81]

1. New vessels at the disc greater than or equal to 1/3 disc area (standard photograph 10A)
2. New vessels at the disc less than 1/3 disc area with pre-retinal or vitreous haemorrhage
3. New vessels elsewhere greater than or equal to 1/2 disc area with pre-retinal or vitreous haemorrhage

2.5 Modified Airlie House classification

Barbara Klein *et al.* described a classification system based on the prognostic significance of retinal features for visual loss. In a study commenced in 1970 191 non-obese subjects with diabetes of at least 5 years' duration and onset before 50 years of age who were taking insulin were followed for 6 years. Stereoscopic colour fundus photographs of seven 30 degree fields in each eye were taken at each of 3 study visits [82]. In multivariate regression analysis individual clinical features were correlated with development of proliferative disease and visual loss. The resulting 'Modified Airlie House Classification' (or Wisconsin system) specified 6 levels of retinopathy for each eye; when both eyes are considered an 11-step grading scheme results. An adaptation of this grading system was used in the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR). This large population-based study aimed to describe the prevalence of complications associated with diabetes and to identify risk factors. A cohort of 996 subjects with younger onset diabetes and 1,370 subjects with diagnosis of diabetes at age 30 or over was first examined between 1980 and 1982 [10,11]; 6 follow-up examinations took place in 1984-86, 1990-92, 1995-96, 2000-01, 2006-07, and 2012-14 [83,84,85].

The Modified Airlie House Classification was revised further for the Early Treatment of Diabetic Retinopathy Study (ETDRS) [86]. Again thirty degree colour photos of 7 fields were graded. Feature specific grading was performed against standard fundus photographs. DR was classified into 13 levels ranging from level 10 (no DR) to 85 (severe vitreous haemorrhage or retinal detachment involving the macula). The ETDRS final retinopathy severity scale is shown in Table 2.1. Definitions of features used in the ETDRS are listed in Appendix 1. This grading system remains the reference standard for classification of DR. Due to complexity its use is almost entirely limited to research studies.

Table 2.1 Early Treatment of Diabetic Retinopathy Study (ETDRS) final retinopathy severity scale (for individual eyes). Reproduced from [70].

Level	Severity	Definition*
10	DR Absent	Microaneurysms and other characteristics absent
14	DR Questionable	HE, SE, or IRMA definite; Microaneurysms absent
15	DR Questionable	Haemorrhage(s) definite; Microaneurysms absent
20	Microaneurysms only	Microaneurysms definite, other characteristics absent
35 [†]	Mild NPDR	One or more of the following: <ul style="list-style-type: none"> • Venous Loops \geq D/1 • SE, IRMA, or VB = Q • Retinal Haemorrhages present • HE \geq D/1 • SE \geq D/1
43	Moderate NPDR	H/Ma = M/4-5 - S/1 or IRMA = D/1-3 (not both)
47	Moderately Severe NPDR	Both L43 characteristics and / or 1 (only) of the following: IRMA = D4-5; H/Ma = S/2-3; VB = D/1
53	Severe NPDR	One or more of the following: <ul style="list-style-type: none"> • \geq 2 of the 3 L47 characteristics; • H/Ma \geq S/4-5 • IRMA \geq M/1 • VB \geq D/2-3
61	Mild PDR	FPD or FPE present with NVD and NVE absent; or NVE = D
65	Moderate PDR	Either of the following: <ol style="list-style-type: none"> 1. NVE \geq M/1 or NVD = D; and VH and PRH = A or Q 2. VH or PRH = D and NVE < M/1 and NVD absent
71	High Risk PDR	Any of the following: <ol style="list-style-type: none"> (1) VH or PRH \geq M/1 (2) NVE \geq M/1 and VH or PRH \geq D/1 (3) NVD = 2 and VH or PRH \geq D/1 (4) NVD \geq M
75	High Risk PDR	NVD \geq M and VH or PRH \geq D/1
81	Advanced PDR: Fundus partially obscured, center of Macular attached	NVD = Cannot Grade, or NVD < D and NVE = Cannot Grade in \geq 1 field and absent in all others; and retinal detachment at center of macular < D
85	Advanced PDR: Posterior Fundus obscured, or center of Macular detached	VH = VS in field 1 and 2; or retinal detachment at center of macular = D
90	Cannot Grade, even sufficiently for L81 or L85	

[†]NPDR levels 35 and above all require presence of microaneurysms

* Severity categories for characteristics graded in multiple fields are of the form (maximum severity/extent), where maximum severity can be absent (A), questionable (Q), definitely present (D), moderate (M), severe (S), or very severe (VS), and extent is the number of fields at that severity level.

DR = diabetic retinopathy; HE = hard exudates; SE = soft exudates (an old term for cotton wool spots); IRMA = intraretinal microvascular abnormalities; NPDR = non-proliferative DR; VB = venous beading; H/Ma = haemorrhages microaneurysms; PDR= proliferative DR; NVE = new vessels elsewhere (>1 disc diameter [DD] from disc); NVD = new vessels disc (within 1 DD of disc margin); FPD = fibrous proliferations disc; FPE = fibrous proliferations elsewhere; VH = vitreous haemorrhage; PRH = preretinal haemorrhage.

The ETDRS was a multicentre randomised controlled trial designed to assess the efficacy of laser therapy (scatter and macular photocoagulation) and aspirin therapy in reducing progression of DR to more advanced DR, the best time to initiate laser treatment and the natural history and risk factors for progression of DR. It commenced in 1979. 3711 subjects were randomised to aspirin or placebo therapy. Additionally one eye in each patient was assigned to immediate laser: one of four combinations of focal and scatter treatment. Aspirin use did not affect progression of DR to high risk characteristics in eyes assigned to deferral of photocoagulation [87]. The ETDRS defined the terms ‘severe non-proliferative diabetic retinopathy (NPDR)’ (level 53) and ‘very severe NPDR’ (Box 2.2). The study showed a statistically significant reduction in severe visual loss in eyes receiving early scatter laser [88]. Severe NPDR was associated with a high rate of progression to high risk characteristics: 15% at 1 year; 56% at 5 years [88]. Very severe NPDR was associated with higher rates of progression: 45% at 1 year; 71% at 5 years [88]. Severe NPDR has subsequently become a widely accepted threshold for initiation of scatter laser treatment (‘the 4-2-1 rule’).

Box 2.2 Severe non-proliferative diabetic retinopathy (NPDR) as defined in the Early Treatment of Diabetic Retinopathy Study (ETDRS) [86]

- Severe NPDR, any one of the following:
 1. 4 quadrants of haemorrhages and microaneurysms (HMa) \geq standard photograph 2A
 2. 2 quadrants of venous changes \geq standard photograph 6A
 3. 1 quadrant of intraretinal microvascular abnormalities (IRMA) \geq standard photograph 8A
- Very Severe NPDR: any 2 of the above

2.6 Classification of diabetic maculopathy

The ETDRS introduced the term clinically significant macular oedema (CSMO)(Box 2.3) [86]. The study demonstrated that macular laser treatment reduced the risk of moderate visual loss (defined as loss of ≥ 15 letters on the ETDRS chart, equivalent

to a doubling of the visual angle) by up to 50% in subjects with CSMO [15]. Macular laser reduced retinal thickening and was associated with an increase in moderate visual gain. On the basis of results from the ETDRS, CSMO is the accepted threshold for macular laser treatment. The ETDRS used clinical examination to grade CSMO in clinical sites but stereoscopic photographs can be used and formed the protocol for grading at the Wisconsin Reading Centre.

Diabetic maculopathy has been classified using the terms focal, diffuse, ischaemic and mixed. Unfortunately these terms are often used without clear definitions. Published definitions for focal and diffuse maculopathy are based on features observed on clinical examination, colour photographs, fluorescein angiography (FA) and optical coherence tomography (OCT), either alone or in combination [89,90,91,92]. Because of the large number of definitions and their inconsistent use, evaluation of published data on prevalence, prognosis and response to treatment of focal and diffuse maculopathy is challenging. Evidence from the ETDRS, which defined these terms according to the source of fluorescein leakage, did not support the concept that classification into focal and diffuse maculopathy is predictive of outcome after laser treatment [89]. Further confusion arises because the term 'focal' is used to describe a technique of applying laser directly to microaneurysms when treating diabetic maculopathy [89]. Some authors have advocated that the use of these terms should be discouraged and replaced by a new vocabulary the features of which correlate with clinical outcomes [93].

Box 2.3 Clinically significant macular oedema (CSMO) as defined in the Early Treatment of Diabetic Retinopathy Study (ETDRS) [86]

1. Retinal oedema within 500µm of the centre of the fovea, or
2. Hard exudates within 500µm of the centre of the fovea if associated with adjacent oedema, or
3. Retinal oedema \geq 1 disc diameter (1500µm) within 1 disc diameter of the centre of the fovea

2.7 Liverpool Diabetic Eye Study grading

The LDES is a large cohort study of subjects in a newly established DR screening programme [9]. The LDES grading scheme is a simplified version of the ETDRS grading system described above [86]. Colour fundus photographs of 3 overlapping 45 degree fields are graded. Retinopathy and maculopathy are classified separately. Modifications of the ETDRS system were made following discussions with the Wisconsin Fundus Photograph Reading Centre, Madison (Professor Simon Harding, personal communication). It was thought that the ETDRS system was too detailed and complex for use in routine clinical care. Therefore modifications were made to simplify the ETDRS system. Alterations were also made to reflect data from the ETDRS on photographic risk factors for progression to PDR and CSMO [70]. Greater weighting was given to venous changes and intra-retinal microvascular abnormalities (IRMA). Reduced weighting was given to CWS. Additionally, presence of exudates outside the macula was excluded from the LDES system and no distinction was made between small haemorrhages and microaneurysms.

The LDES grading scheme includes 10 levels for retinopathy and 6 for maculopathy (plus the option of 'ungradeable' for either category). Level of retinopathy is determined for each eye and a grade assigned based on the worse eye. Similarly, each eye is graded for maculopathy and the grade of the worse eye assigned to that subject. The macula is defined as a circular zone centered on the fovea, whose radius is the distance from the centre of the fovea to the temporal margin of the optic disc. In the LDES, retinal thickening is not determined directly for classification of maculopathy (this would require the use of clinical examination or stereoscopic photos). A grading system based on the presence of exudates is used. Levels of retinopathy and maculopathy in the LDES are shown in Table 2.2.

Table 2.2 Levels of retinopathy and maculopathy in the Liverpool Diabetic Eye Study [94]

Level	Definition
Retinopathy	
10	No retinopathy
12	Questionable
20	Haemorrhages or microaneurysms < ETDRS standard photograph 2A †
30	Haemorrhages or microaneurysms > ETDRS standard photograph 2A, and/or 1-6 cotton wool spots
40	Haemorrhages/ microaneurysms > ETDRS STD 2A and/or > 6 cotton wool spots; and/or 1 quadrant venous changes; and/or IRMA < ETDRS STD 8A
50	IRMA > ETDRS STD 8A and/or 2 or more quadrants venous changes and/or pre-retinal haemorrhage in absence of proliferation
60	Fibrovascular proliferation and/or proliferative retinopathy
70	Diabetic Retinopathy Study high risk characteristics
71	Tractional retinal detachment
72	No fundal view due to vitreous blood
90	Ungradeable due to any other reason e.g. media opacity
Maculopathy	
0	No maculopathy
1	Questionable: < 50% certainty of presence of exudate
2	Exudate >1 disc diameter (DD) from fixation
3	Circinate ring of exudates within macula >1 disc area in size but not within 1 DD of fixation
4	Exudates within 1 disc diameter of fixation and/or presence of clinically significant macular oedema
8	Exudates due to other diseases e.g. vein occlusion, choroidal neovascularisation
90	Ungradeable

† Definition of any diabetic retinopathy: ≥ 1 haemorrhages of microaneurysms (HMa) in either eye. Flame shaped haemorrhages associated with hypertension are discounted.
ETDRS STD = Early Treatment of Diabetic Retinopathy Study Standard Photograph
IRMA = Intraretinal Microvascular Abnormalities

2.8 Comparison of LDES grading with the WESDR and ETDRS systems

Both the ETDRS and the WESDR used 7-field 30-degree colour stereoscopic photography [86]. In contrast the LDES used 3-field 45-degree non-stereoscopic photographs [9]. The former photographic protocol images a greater proportion of the retina but still covers only 17% of the total retinal area [86]. 30-degree images provide greater magnification than 45-degree images. Stereoscopic images allow the detection of retinal thickening and oedema. However, 7-field stereoscopic photography is technically difficult, time consuming and expensive: important in the context of a screening program and a large epidemiological study [95].

The LDES grading scheme is a simplified version of the ETDRS system [86]. The LDES scheme has been validated for progression to sight threatening diabetic retinopathy (STDR; defined below) for persons with type 1 [96] and type 2 diabetes [9].

Mapping of LDES levels onto ETDRS grades provides additional (indirect) evidence for risk of progression. The LDES moderate pre-proliferative retinopathy grade (level 40) is roughly equivalent to ETDRS level 43 with risk at 1 year of development of PDR and high risk PDR of 11.9% and 3.2%, respectively [70,97]. Unlike the ETDRS scheme the LDES scheme has not been validated in terms of the degree of risk of progression to visual loss or blindness; a limitation of this grading methodology.

2.9 Classification of maculopathy in the LDES

In clinical practice diagnosis of sight-threatening maculopathy depends upon examination by stereoscopic slit lamp biomicroscopy. Research studies have utilised slit-lamp biomicroscopy, stereoscopic colour photographs and stereoscopic FA to detect the condition [98]. Screening for DR using non-stereoscopic photography relies on surrogate markers, chiefly exudates. The LDES classifies maculopathy according to the pattern of exudates and their distance from the centre of the fovea (Table 3.2). Evidence from the ETDRS shows that presence of an exudate within a disc diameter from the centre of the fovea has a sensitivity of 94% for detection of CSMO but a specificity of only 54% [99]. The LDES grading scheme can be criticised for an inability to directly detect retinal thickening and a dependence on surrogate markers which may result in an underestimate of the frequency of retinal thickening.

2.10 Sight threatening diabetic retinopathy

The definition of STDR is not universally agreed upon. A number of definitions have been proposed [100,101,102,103]. In the LDES, STDR was defined as any of the following: moderate pre-proliferative retinopathy or worse (retinopathy level 40-71+); macular exudates in a circinate pattern or within one disc diameter of the

foveal centre, or CSMO (maculopathy level 3-4). Sight threatening retinopathy is a subset of STDR defined in the LDES as moderate pre-proliferative retinopathy or worse (level 40-71+). Sight threatening maculopathy is a subset of STDR defined in the LDES as macular exudates in a circinate pattern or within one disc diameter of the foveal centre, or CSMO (level 3-4) [9].

2.11 Simplified grading schemes: the English National Screening Programme

A National Screening Programme for diabetic eye disease has been developed in the UK. A screening program requires a grading system which facilitates appropriately prioritised referrals to a treatment service but does not require the many classification levels necessary for detailed monitoring of DR in clinical practice or natural history research studies. Structured discussions of an expert panel took place between 2000 and 2002 to review existing grading schemes and design a suitable classification. The aims of the committee were to maximise detection of STDR (sensitivity) whilst minimising false positive referrals to the hospital eye service (specificity). The principles employed in this simplified grading scheme were: separate grading of retinopathy and maculopathy; minimum number of steps; no lesion counting; compatibility with central monitoring; expandable for established more complex systems and for research; to allow precise quality assurance at all steps (in excess of the Exeter Standards for DR screening [104]).

Table 2.3 details the English National Screening Programme (ENSP) grading scheme [105]. The ENSP originally defined STDR as: pre-proliferative retinopathy or worse (R2), sight-threatening maculopathy (M1) and/or presence of photocoagulation (P1) (more recently presence of photocoagulation has been removed from the ENSP definition of STDR). These levels equate to referable retinopathy or maculopathy. This is the same as the LDES definition for STDR except for the inclusion of presence of haemorrhages/microaneurysms (HMa) within 1 disc diameter of the centre of the fovea and a visual acuity of 6/12 or worse. The National Screening Committee (NSC) decided that the significance of CWS was unclear. Therefore CWS were

included in the grading protocol only as a marker to indicate that a grader should search for other signs of pre-proliferative DR.

Table 2.3 Levels of retinopathy in the English National Screening Programme grading scheme. Reproduced from [105].

Retinopathy (R)		
Level 0	None	
Level 1	Background	Microaneurysm(s), retinal haemorrhage(s) ± any exudate
Level 2	Pre-proliferative	Venous beading, Venous loop or reduplication, Intraretinal microvascular abnormality (IRMA) Multiple deep, round or blot haemorrhages (CWS-careful search for above features)
Level 3	Proliferative	New vessels on disc (NVD) New vessels elsewhere (NVE) Pre-retinal or vitreous haemorrhage Pre-retinal fibrosis ± tractional retinal detachment
Maculopathy (M)		
M1		Exudate within 1 disc diameter (DD) of the centre of the fovea Circinate or group of exudates within the macula Retinal thickening within 1DD of the centre of the fovea (if stereo available) Any microaneurysm or haemorrhage within 1DD of the centre of the fovea only if associated with a best VA of ≤ 6/12 (if no stereo)
Photocoagulation (P)		
		Focal /grid to macula Peripheral scatter
Unclassifiable (U)		
		Ungradeable/unobtainable

2.12 Comparison of the LDES and ENSP systems

Both the LDES and ENSP systems require separate grading for retinopathy and maculopathy. In both systems the principle outcome is presence or absence of STDR. When used for screening purposes STDR equates to referable retinopathy or maculopathy. The LDES is more detailed, has more levels and, unlike ENSP, relies on counting of DR lesions. The definition of sight threatening maculopathy is very

similar in both systems except that ENSP includes presence of microaneurysms or haemorrhages within 1DD of the centre of the fovea if associated with a best VA of $\leq 6/12$ as a referral criteria. The significance of HMa within 1DD of the centre of the fovea in the absence of other lesions is unclear (Simon Harding, personal communication). An unpublished study from 2005 (Paul Dodson, personal communication) indicated that 10% of persons with HMa within 1DD of the centre of the fovea (and no other lesions) progress to exudative maculopathy within 12 months. No subjects in this study had progressed to CSMO in this time period.

Like the LDES the ENSP grading scheme has not been directly validated for risk of development of visual loss. However, retinopathy level R2 maps to ETDRS level 43 described in Section 2.8 above. As described above, evidence from the ETDRS demonstrates that an exudate within a disc diameter from the centre of the fovea has a sensitivity of 94% for detection of CSMO [99]. Shortly after the introduction of the ENSP scale a very similar Scottish Diabetic Retinopathy Grading Scheme was produced [106]. Table 2.4 shows a comparison table of grading schemes used in screening programmes together with the international DR severity scale introduced in Section 2.12.

Table 2.4 Comparative table of grading classifications and suggested management of DR. ETDRS = Early Treatment of Diabetic Retinopathy Study; LDES = Liverpool Diabetic Eye Study; ENSP= English National Screening Programme for Diabetic Retinopathy; SDRGS = Scottish Diabetic Retinopathy Grading System; ICDRSS = International Clinical Diabetic Retinopathy Disease Severity Scale. Adapted from [98]

ETDRS	LDES	ENSPDR	SDRGS	ICDRSS
10	10 Annual screen	R0 No DR Annual rescreen	1 No DR Annual rescreen	No DR Annual screen
20	20 Annual screen	R1 BDR Annual screen	2 Mild BDR Annual screen	Mild NPDR Annual screen
35	30 Screen 6 monthly	Inform physician	3 Moderate BDR Early rescreen	Moderate NPDR Refer
43	40	R2	4 Severe BDR	
47	Refer	Pre-proliferative DR	Refer	
53	50 Refer Consider laser	Refer	5 Very severe BDR	Severe NPDR Consider laser
61, 65	60 Laser	R3 PDR Fast-track refer	6 PDR early	PDR Urgent laser
71, 75	70		7 PDR – HRC	PDR HRC
81, 85	Urgent laser		8 Advanced	Urgent laser

DR = diabetic retinopathy; BDR = background diabetic retinopathy; NPDR = non-proliferative DR; PDR = proliferative DR; HRC = high-risk characteristics.

2.13 Simplified grading schemes: an international clinical severity scale

As described above the ETDRS system is regarded as the reference standard for grading DR. This system has more levels than may be required in clinical care and the definitions of the levels are detailed, require comparison with standard photographs and are difficult to remember. In order to provide a framework for improved communications between primary care physicians, endocrinologists,

ophthalmologists, and other eye care providers an international working group was convened by the American Academy of Ophthalmology (AAO) in 2002 to produce a simplified grading system for clinical use [107]. In order that the scheme was based on evidence of the natural history of DR and could be mapped to progression to visual loss, levels were based on the ETDRS system. Risks of progression and vision loss can therefore be taken from the ETDRS and the WESDR.

The resulting International Clinical Diabetic Retinopathy Severity Scales (ICDRSS) classification system has 5 levels for DR: 3 'low risk' stages, a fourth stage of severe NPDR, and a fifth stage of proliferative DR (Table 2.5). Level 2 corresponds to ETDRS level 20; level 3 to ETDRS level 35 through 47. Level 4 corresponds to ETDRS level 53 (severe NPDR); the '4-2-1 rule' a widely agreed threshold for scatter laser treatment. Diabetic macular oedema (DMO) is graded as present or absent and then further classified according to distance from the centre of the macula. This 2 tiered system reflects the reality that many examiners may not have the training or equipment (stereoscopic examination techniques) required for reliable detection of retinal thickening.

The ICDRSS classification system is primarily designed for use in the United States where standard practice is to refer to an Ophthalmologist at Level 3 retinopathy. This scheme is not suitable for health systems in which there is a need to detect sight threatening DR which is likely to require treatment. An obvious criticism of the ICDRSS scale for maculopathy is that the distance of exudates in each category from the centre of the fovea is not explicitly stated. This leads to a degree of subjectivity in grading. The sensitivity and specificity of this system for detection of DR and DMO has not been validated against the reference standard ETDRS system in high-quality clinical studies. The impact of the use of this system on medical treatment of those with diabetes (through greater communication between primary care physicians, endocrinologists and ophthalmologists), on the number of persons receiving appropriate timely treatment for DR and on the degree of visual loss prevented in a variety of settings has yet to be fully evaluated.

Table 2.5 The American Academy of Ophthalmology International Clinical Diabetic Retinopathy Severity Scales (ICDRSS) for retinopathy and maculopathy. DMO = diabetic macular oedema. Reproduced from [107].

Retinopathy

Level 1	No apparent retinopathy	No abnormalities
Level 2	Mild non-proliferative diabetic retinopathy	Microaneurysms only
Level 3	Mod non-proliferative diabetic retinopathy	More than just microaneurysms but less than severe nonproliferative diabetic retinopathy
Level 4	Severe non-proliferative diabetic retinopathy	Any of the following: more than 20 intraretinal haemorrhages in each of 4 quadrants; definite venous beading in 2+ quadrants; Prominent intraretinal microvascular abnormalities in 1+ quadrant(s) <i>And no</i> signs of proliferative retinopathy
Level 5	Proliferative diabetic retinopathy	One or more of the following: neovascularization, vitreous/preretinal haemorrhage

Maculopathy

DMO apparently absent posterior pole	No apparent retinal thickening or hard exudates in
DMO apparently present post. pole	Some apparent retinal thickening or hard exudates in

If diabetic macular oedema present:

- Mild DMO: Some retinal thickening or hard exudates in posterior pole but distant from the centre of the macula
- Moderate DMO: Retinal thickening or hard exudates approaching the centre of the macula but not involving the centre
- Severe DMO: Retinal thickening or hard exudates involving the centre of the macula

2.14 Definition of ‘any diabetic retinopathy’

The definition of ‘any DR’ is important because a number of high quality studies have demonstrated retinal lesions meeting study specific criteria for DR in subjects defined as not having diabetes. The Diabetes Prevention Program (DPP) [108] studied a cohort of 3819 subjects with elevated fasting glucose (5.3–6.9 mmol/l) and impaired glucose tolerance but with no history of diabetes and not meeting WHO criteria for the diagnosis of diabetes. Fundus photography was performed in

a selected subgroup of subjects and photographs graded at the Fundus Photography Reading Centre at the University of Wisconsin. After mean follow-up of 5.6 years, retinopathy consistent with DR (defined as presence of any microaneurysms) was detected in 12.6% of subjects who developed diabetes during the study and 7.9% of subjects who remained without diabetes [108]. This study demonstrates that retinopathy may start in the state now considered 'pre-diabetes' and that DR, as currently defined, may be less specific to diabetes than previously thought. To further complicate the picture resolution of type 2 diabetes is reported (for example following bariatric surgery [109]). This scenario may be associated with presence of retinopathy but not diabetes.

The Blue Mountains Eye Study of 2335 subjects without diabetes, as evaluated by the absence of a history of diabetes and normal fasting plasma glucose, demonstrated a 9.7% 5-year cumulative incidence of developing retinal microaneurysms, haemorrhages, CWS or exudates [110]. In this study retinal changes were not associated with high blood pressure or fasting plasma glucose. Retinopathy has also been reported in subjects with normal glucose tolerance. A cross sectional study of Pima Indians (a population with an extremely high prevalence of diabetes) demonstrated retinal lesions consistent with DR (using the same criteria as the DPP) in 12% of subjects with impaired glucose tolerance and 3% with normal glucose tolerance [111]. It is clear that retinal vascular lesions are not uncommon regardless of glycaemic status. The LDES grading scheme draws no distinction between dot haemorrhages and microaneurysms increasing the possibility that retinal lesions detected and defined as DR may not be specific to diabetes.

2.15 The role of optical coherence tomography and fluorescein angiography

The use of imaging technology has aided understanding of retinal vascular diseases including DR. However, the majority of grading schemes have relied on clinical characteristics observed on colour fundus photographs and/or clinical examination.

The ETDRS included use of stereoscopic FA to assess characteristics including capillary loss, capillary dilatation, arteriolar abnormalities, abnormalities of the retinal pigment epithelium, cystoid changes and leakage. A classification system based on comparison with standard photographs was produced [112].

Fluorescein leakage (particularly diffuse leakage), capillary loss and dilatation at baseline were associated with severity of DR assessed on colour fundus photographs and with likelihood of progression to PDR during follow-up [113]. FA was also used to guide laser treatment of macular oedema [89].

OCT is an imaging technique which uses low coherence interferometry to capture micrometer-resolution images of biological tissue. OCT has revolutionised understanding of macular pathology including DMO and the vitreo-macular interface. Several studies have produced grading schemes based principally on morphological characteristics of DMO on OCT [114, 115]. Bolz et al [116] recently published a more comprehensive grading scheme based on both OCT and FA findings. The protocol contained four areas of interest: subretinal fluid; area of oedema; vitreo-retinal interface abnormalities; and source of leakage. The authors suggested that their grading scheme may facilitate improved choice of appropriate therapies for DMO e.g. choice between macular grid laser and anti-VEGF therapies. However, the treatment strategies appropriate for the different categories of DMO in this grading scheme are not well defined. Validation studies are required in relation to visual function and disease prognosis. FA and OCT imaging are not widely available in Sub-Saharan Africa. Grading schemes based on these imaging techniques are not yet applicable in this region.

2.16 Automated grading of DR

Grading DR by clinical examination or by manual inspection of fundus images requires trained professionals and is time consuming. It is therefore expensive and a major limitation on the cost effectiveness of DR screening programmes. Automated systems have been developed to detect and grade DR from fundus photographs. The process of analysing retinal images requires identification of

basic anatomical structures, recognition of pathological features, and extraction and classification of lesions. A number of computer algorithms have been developed for automated segmentation of anatomical features (optic disc, blood vessels and the fovea) and abnormal lesions (microaneurysms and exudates) [117,118].

The 'Retmarker' automated grading system has been tested according to its ability to accurately assign fundus photographs to 'disease' or 'no disease' categories. This system has been tested on fundus photographs from a screening service and compared to manual grading for a dataset which included 33,535 patient episodes. The software achieved between 95.0 and 98.1% and between 97.6% and 100% sensitivity for disease/no disease in subjects with referable retinopathy and PDR, respectively [119,120,121]. It was estimated that use of this system would result in a workload reduction for manual graders of between 38 and 46%. The authors concluded that use of this automated system could safely reduce the workload of manual graders with large potential cost savings. A prospective observational study is currently being conducted to quantify screening performance, diagnostic accuracy and cost-effectiveness of automated primary grading of fundus images in the ENSP with results expected in 2015 (Mr Adnan Tufail, Moorfields Eye Hospital, personal communication). It is likely that automated grading will play an increasing role in DR research studies and screening programmes with significant cost implications.

2.17 Chapter summary

There are many grading schemes for DR. The reference standard is the ETDRS scale: levels are validated for progression of retinopathy and visual loss as described in the WESDR and the ETDRS. Other grading schemes including the LDES scale map to ETDRS levels providing an evidence base for referral and treatment thresholds. Detailed and validated grading schemes for DR facilitate studies of the determinants of severity and progression of DR. In Chapter 3 I review the existing evidence on this topic.

Chapter 3. Determinants of Severity and Progression of Diabetic Retinopathy

3.1 Aims of chapter

In this chapter I review and appraise the existing evidence on the determinants of severity and progression of diabetic retinopathy (DR).

3.2 Introduction

The macro- and micro-vascular complications of diabetes were once thought to be an inevitable consequence of the disease. Major epidemiological studies since the 1970s have informed understanding of the pathophysiology of diabetes and DR and are key to understanding primary prevention of retinopathy. The vast majority of these studies were performed in Europe and North America. In the absence of good epidemiological evidence from sub-Saharan Africa (SSA), results are generalised to this region. The evidence presented below is a starting point for investigation of determinants of severity and progression of DR in Southern Malawi.

3.3 Outcome measures in studies of epidemiology and treatment

Comparison between individual studies of DR is impeded by use of a number of different classification systems and a variety of different methods to assess the retina. Classification of DR is described in Chapter 2 of this thesis. The reference standard for assessment uses modifications of the Airlie House classification [86] based on grading of seven stereoscopic, 30 degree photographs. Each eye receives a score and the grades for both eyes are combined on a stepped scale (usually according to grade in the worst eye). Maculopathy is graded separately.

Progression is usually defined in clinical trials and epidemiological studies as a 2 or 3 step increase in DR grade from baseline (eg. 2 step = either 2 step progression in one eye or 1 step progression in both eyes). Other surrogate markers of

progression include occurrence of vitreous haemorrhage, need for scatter laser photocoagulation and need for vitreoretinal surgery.

Visual acuity (VA) is the preferred primary outcome for studies assessing treatments of DR. The main disadvantage of this approach is that other pathologies (e.g. cataract) and additional interventions (e.g. cataract surgery) may adversely or beneficially affect vision independent of effect on DR. The reference standard for measurement of VA is the Early Treatment Diabetic Retinopathy Study (ETDRS) chart read at 4m. Advantages over the conventional Snellen chart include reduced effect of crowding as each line has 5 letters. Loss of 15 letters (3 lines) is equivalent to doubling of the visual angle and was termed in the ETDRS studies as 'moderate visual loss' (MVL).

3.4 Overview of determinants of severity and progression

Traditionally, determinants of DR severity and progression have been classified into modifiable and non-modifiable risk factors. Epidemiological studies as well as intervention studies have informed our understanding of modifiable factors. Glycaemic control and blood pressure are the variables which have been most thoroughly studied. More recently, intervention studies have addressed the role of lipids in DR. There is little data on the contribution of anaemia and infections including HIV. Large, high-quality cohort and cross sectional studies have reported the effects of non-modifiable risk factors. The effect of duration of diabetes since diagnosis is well studied; a number of authors have estimated the mean duration of diabetes prior to diagnosis in groups of subjects with type 2 disease based on progression of DR data. The role of ethnicity in DR prevalence and progression is unclear. Genetic studies will offer further insights into the pathophysiology of DR and open the possibility of individually targeted therapies based on genotype.

3.5 Modifiable risk factors

3.5.1 Measurement of glycaemic control

Glycaemic control is an important determinant of DR severity and progression as described in Section 3.5.2 below. Measures of glycaemic control in diabetes include fasting plasma glucose (FPG) and glycated haemoglobin (HbA1c). HbA1c is a form of haemoglobin produced in a non-enzymatic glycation pathway by exposure of haemoglobin to plasma glucose. Measurement involves 2 processes. The first separates the fractions of haemoglobin by either affinity chromatography (AC), ion exchange chromatography (IEC) or capillary electrophoresis (CE). The second measures the concentration of HbA1c based on a specific chemical reaction to the glycated N-terminal of the haemoglobin beta-chain. Tests are either immunochemical or enzymatic. Total haemoglobin concentration is also measured allowing the proportion of glycated haemoglobin to be determined. Although different analytes are measured by these assays, testing is standardised by the reference measurement procedure of the International Federation of Clinical Chemistry (IFCC).

Both FPG and HbA1c measurements show a relationship to risk of retinopathy [122]. Measurement of HbA1c is the standard method for monitoring glycaemic control in Europe and North America. More recently, recognising that testing is now highly standardised, the American Diabetes Association have approved its use for diagnosis of diabetes [123]. The advantages of HbA1c over FPG include convenience (no fasting required), fewer day-to-day fluctuations during episodes of illness or stress, and evidence to suggest greater pre-analytical stability [123]. Disadvantages of HbA1c include greater cost and the fact that testing requires strict quality management. Accredited laboratories must demonstrate internal control procedures and participate in an external quality assessment programme. Measurement of HbA1c is affected by haemoglobin variants (including Hb S & C prevalent in black Africans), anaemia (more prevalent in the developing world) and HIV infection (highly prevalent in many countries in sub-Saharan Africa). For these

reasons FPG, not HbA1c, is the standard for measurement of glycaemic control in most countries in sub-Saharan Africa. To my knowledge HbA1c has not been validated in populations in East Africa.

3.5.2 Glycaemic control

Two pivotal randomised controlled trials (RCTs) demonstrated the importance of good glycaemic control in reducing the incidence and progression of retinopathy in diabetes: the Diabetes Complications and Control Trial (DCCT) in type 1 diabetes and the United Kingdom Prospective Diabetes Study (UKPDS) in type 2 diabetes.

DCCT

The DCCT was conducted in 29 centres in the United States and Canada between 1983 and 1989. 1441 subjects with type 1 diabetes of greater than 1 and less than 15 years duration (726 with no retinopathy and 715 with background DR at baseline) were randomised to either intensive or conventional glycaemic control (mean HbA1c achieved 7.2% and 9.1%, respectively). Mean duration of follow-up was 6.5 years [12,124,125,126].

For subjects with no DR at baseline the incidence of retinopathy (defined as a change of three steps on the ETDRS scale that was sustained over a six month period) was 76% (95% CI 62-85) lower in the intensive therapy arm than the conventional arm [12]. For subjects with background retinopathy at baseline intensive therapy reduced the risk of retinopathy progression by 3 steps (or more) by 54% (95% CI 39-66). Intensive therapy reduced the risk of severe non-proliferative DR or proliferative retinopathy (PDR) by 47% and that of treatment with laser photocoagulation by 56% [12]. Figure 3.1 shows cumulative incidence of DR progression in subjects with and without retinopathy at baseline. The authors concluded that intensive control was most effective when started before retinopathy was detectable.

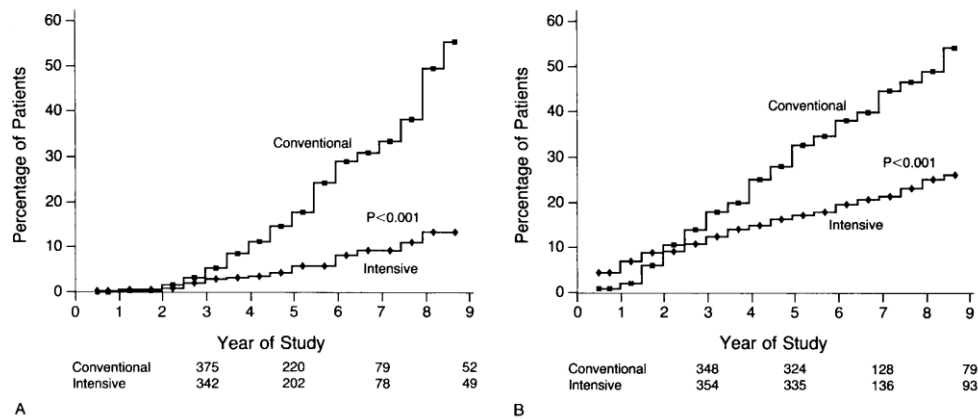


Figure 3.1 Cumulative incidence of sustained 3 step progression of retinopathy in subjects with type 1 diabetes receiving intensive or conventional therapy. A: subjects with no retinopathy at baseline. B: subjects with background DR at baseline. Numbers of subjects in each therapy group who were evaluated at years 3,5,7 and 9 are shown below the graphs. Taken from the DCCT [12].

In the DCCT, intensive therapy reduced the incidence of microalbuminuria by 39%, albuminuria by 54% and neuropathy on clinical examination by 60% [12]. The absolute risks of nephropathy and retinopathy were proportional to the mean HbA1c level over the follow-up period preceding each event. Mortality did not differ significantly between the intensive and conventional therapy arms. However, the incidence of severe hypoglycaemia was 3 times higher in the intensive arm. A person not involved in the trial was killed in a road traffic accident after being hit by a motorbike ridden by a subject in the intensive arm who had an episode of hypoglycaemia. More weight gain was observed in the intensive therapy arm.

Follow-up of the DCCT cohort continued annually as part of the Epidemiology of Diabetes Interventions and Complications (EDIC) study [127,128]. During this study glycaemic control no longer differed substantially between the original 2 treatment arms of the trial: at one year the difference was 0.4% (HbA1c 8.3% in the former conventional therapy arm and 7.9% in the former intensive arm, $p < .001$) and by 5 years the difference was non-significant. In analyses using the DCCT final retinopathy grade as a new baseline, at 4 years of EDIC further progression of DR was between 66 to 77% less (depending on the measure used) in the former

intensive arm than the former conventional arm [127]. This phenomenon has been termed 'metabolic memory'. Cumulative incidence of 3 step progression was still significantly less at 7 years [127] and 10 years [128]. Significantly fewer former intensive subjects than former conventional subjects required laser treatment during EDIC [128]. The EDIC study demonstrated a reduction in cardiovascular disease in the former intensive group which had not been apparent in the DCCT [129].

The overall results of the DCCT/EDIC were interpreted by the authors to show that the benefits of intensive glycaemic control take some years to manifest but subsequently persist beyond the period of intervention. The authors hypothesised that the predictive association of risk of DR with preceding mean HbA1c was a causal relationship and that total glycaemic exposure determines the degree of retinopathy observed at any one time. A HbA1c target level of 7.0% or less was suggested [127] although the authors acknowledged the risks of increased hypoglycaemic events and excess weight gain with intensive therapy regimes [130,131].

The DCCT did not define the optimum methods to achieve good glycaemic control while minimising adverse events; subsequent studies would explore the benefits and risks of treatment regimens aimed at reducing mean HbA1c further. While the DCCT/EDIC analyses were adjusted for a number of baseline physiological parameters there was no adjustment for use of medications other than hypoglycaemic agents (e.g. aspirin and angiotensin-converting enzyme inhibitors) which is a potential confounder. The DCCT/EDIC studies commenced over 30 years ago and results are increasingly difficult to apply to current practice. Many aspects of diabetes care have changed markedly over this time. In particular management of blood pressure was generally much worse than at present. These caveats notwithstanding concepts such as metabolic memory are still highly relevant.

UKPDS

The UKPDS was conducted between 1977 and 1997. 3867 subjects with newly diagnosed type 2 diabetes (median age 54 years; IQR 48-60 years) who, after 3 months diet treatment had a fasting plasma glucose concentration of 6.1-15.0 mmol/L, were randomly assigned to intensive glycaemic control with a sulphonylurea or insulin, or conventional therapy with diet [132,133,134,135]. Mean HbA1c levels achieved were 7.0% (6.2–8.2) and 7.9% (6.9–8.8), respectively. Median follow-up was 10.0 years (IQR 7.7–12.4). Subjects in the intensive treatment arm had a significant 25% risk reduction in microvascular endpoints. Most of this reduction was due to fewer subjects requiring retinal laser treatment. The UKPDS investigated surrogate endpoints, including 2 step progression of DR, at visits at 3 year intervals. Subjects in the intensive treatment arm demonstrated a 34% reduction in incidence of DR and 17% lower rate of 2 step progression (a difference which was significant even when subjects who received retinal laser were excluded). Each 1% reduction in HbA1c gave a 31% reduced risk of incidence or 2 step progression of DR.

Relatively few subjects in the UKPDS developed late complications of DR such as vitreous haemorrhage or blindness. This may be because the follow-up period was not long enough. A more likely explanation is that subjects were examined regularly and received laser photocoagulation as necessary. Despite this the intensive arm showed a 16% reduction in legal blindness. With regard to macrovascular endpoints the UKPDS showed evidence of a 16% risk reduction for myocardial infarction, which included non-fatal and fatal myocardial infarction and sudden death. This difference was not statistically significant by conventional criteria: $p=0.052$. It is possible that this difference may have been greater given a longer duration of follow-up. Interpretation of this outcome is complicated by the multifactorial nature of cardiovascular disease. Diabetes-related mortality and all-cause mortality did not differ between the intensive and conventional groups. However, the study was not sufficiently powered to exclude a beneficial effect on mortality.

The number needed to treat to prevent one subject developing any of the single trial endpoints (either micro or macrovascular) over 10 years was 19.6 subjects (95% CI 10–500). The ‘complication free interval’ (defined as the time at which 50% of subjects had at least one diabetes related trial endpoint) was 14.0 years in the intensive arm and 12.7 years in the conventional arm ($p=0.029$). As in the DCCT the proportion of subjects experiencing hypoglycaemic episodes was significantly higher in the intensive arm. In subjects treated with insulin each year, 3% had a major episode and 40% a minor or major hypoglycaemic episode. Subjects in the intensive arm also demonstrated significantly increased weight: mean 3.1 kg (99% CI –0.9 to 7.0, $p<0.0001$) at 10 years.

Importantly the UKPDS showed that clinical benefit could be gained at lower HbA1c values than DCCT. The UKPDS compared a difference in HbA1c between intensive and conventional arms of 0.9% (7.0 vs 7.9%) over 10 years. This is smaller than the difference compared in the DCCT: 1.9% (7.2 vs 9.1%). The DCCT studied younger subjects with type 1 diabetes and used slightly different (mainly surrogate) outcome measures. With this caveat, comparison of DCCT and UKPDS results suggests that the benefits of HbA1c reduction on risk of progression of microvascular disease are proportional: 21% for retinopathy in UKPDS and 63% in the DCCT, and, for albuminuria, 34% and 54% respectively.

ACCORD and ADVANCE

The DCCT and UKPDS demonstrated unequivocal reductions in ocular morbidity with improved glycaemic control. However, the potential benefits of tighter control remain controversial. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) and Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) were large RCTs of glycaemic control to levels lower than the DCCT and UKPDS. In the ACCORD study 10,251 subjects with type 2 diabetes who were at high risk for cardiovascular disease were randomised to either intensive or standard glycaemic control (target $<6.0\%$ or 7.0 to 7.9%; at one year achieved mean HbA1c were 6.4% and 7.5%, respectively). Of these participants 5518 with dyslipidaemia were randomised to either intensive or

standard therapy: 160 mg daily of fenofibrate plus simvastatin or placebo plus simvastatin. The remaining 4733 subjects were randomised to either intensive or standard therapy for systolic blood-pressure control: target <120 or <140 mm Hg [136,137]. A sub-group of 2856 subjects was evaluated at 4 years for the effects of these interventions on the progression of DR by 3 (or more) steps on the ETDRS scale or development of DR necessitating laser photocoagulation or vitrectomy.

At 4 years follow-up, progression of DR was seen in 7.3% of the intensive group and 10.4% of the standard group (adjusted odds ratio 0.67; 95% CI 0.51 - 0.87; $p=0.003$). In the ACCORD Eye study there was a non-significant trend towards reduced MVL (23.8% vs 26.3%; hazard ratio 0.88; 95% CI 0.77-1.01; $p=0.06$). However, in the entire ACCORD population there was a significant reduction in MVL in the intensive group (19.1% vs 20.7% with standard therapy; hazard ratio, 0.91; 95% CI, 0.83 to 1.00; $P = 0.047$)[138]. The authors concluded that intensive glycaemic control reduced the rate of progression of DR and reduced visual loss (even in a population receiving regular eye examination and with access to treatment for DR).

Unfortunately the ACCORD trial showed a significantly increased risk of having a hypoglycaemic event that necessitated assistance in the intensive arm. Most worryingly the intensive regime was associated with an increase in all-cause mortality after mean 3.5 years follow-up. This led to the premature cessation of the glycaemic trial. The study may therefore have underestimated the reported effect of glycaemic treatment on DR. More importantly though ACCORD demonstrates real risks associated with intensive treatment strategies. The ACCORD population were older than those of the DCCT and UKPDS and pre-selected to be at high risk of cardiovascular disease. Results from the trial regarding risks should be generalised to all patients with type 2 diabetes with caution.

The ADVANCE study randomly assigned 11,140 patients with type 2 diabetes to either intensive (gliclazide plus other drugs) or standard control. After median follow-up 5 years mean HbA1c achieved was 6.5% and 7.3%, respectively [139]. A subgroup of 1241 subjects in the ADVANCE Retinal Measurements (AdRem) study underwent retinal photography and ETDRS grading of DR [140]. In the main study

intensive control reduced the incidence of major microvascular events (hazard ratio 0.86; 95% CI, 0.77 to 0.97; $p=0.01$) and of combined major macrovascular and microvascular events (hazard ratio 0.90; 95% CI 0.82-0.98; $p=0.01$). These results were mainly due to a reduction in incidence of nephropathy.

In the main study there was no significant difference between the 2 arms in the number of subjects undergoing retinal photocoagulation. However, the absolute numbers requiring laser treatment were low thus limiting the power of the study to detect a difference. In the AdRem sub-study there was no significant reduction in development or progression of DR or maculopathy. There were no significant differences between the intensive and standard arms with regard to major macrovascular events, death from cardiovascular cause or death from any cause. Intensive control was associated with an increased risk of severe hypoglycaemia (hazard ratio 1.86; 95% CI 1.42-2.40; $p<0.001$) and an increased risk of hospitalisation.

Steno 2

Trials assessing multifactorial interventions have informed understanding of the effects of glycaemic control on DR. The Danish Steno 2 study randomised 160 subjects with type 2 diabetes and persistent microalbuminuria to an intervention comprising tight glucose control, the use of renin–angiotensin system blockers, aspirin, and lipid-lowering agents and behavioural modification or standard care. The mean treatment period was 7.8 years and subsequent follow-up continued for a mean of 5.5 years. The intensive group showed reduced risk of retinal photocoagulation (relative risk 0.45; 95% CI 0.23-0.86; $p=0.02$), all-cause mortality (hazard ratio 0.54; 95% CI 0.32-0.89; $p=0.02$), death from cardiovascular causes (hazard ratio, 0.43; 95% CI 0.19-0.94; $p=0.04$) and of cardiovascular events (hazard ratio, 0.41; 95% CI 0.25-0.67; $p<0.001$) [141]. Few major side effects were reported. This study showed large relative and absolute improvements in microvascular and macrovascular outcomes. It was not designed to identify the relative contributions of the individual elements in the intervention package. The Steno 2 study is one of the most important studies on which modern diabetes management is based. Box

3.1 lists the targets for modifiable risk factors and behavioural changes that were set in the study.

Box 3.1 Targets for modifiable risk factors and lifestyle changes set by the Steno 2 study [141]

- Glycosylated haemoglobin (HbA1c) < 6.5%
- Blood pressure < 130/80 mmHg
- Total cholesterol < 175 mg/dl
- Triglycerides < 150 mg/dl
- Angiotensin-converting enzyme (ACE) inhibitor (regardless of blood pressure): equivalent to captopril 50mg twice daily
- Aspirin 150 mg daily to patients with a history of cardiovascular disease
- Reduction in intake of dietary fat: total daily intake of fat less than 30% of total daily energy intake, and intake of saturated fatty acids of less than 10% of total daily energy intake
- Regular exercise: at least 30 min three to five times a week
- Smoking cessation
- A dietary supplement consisting of vitamins C (250 mg daily) and E (100 mg daily), folic acid and chromium picolinate

Therapeutic issues in glycaemic control

A goal of HbA1c <7% is suitable for the majority of persons with diabetes [142]. Certain patients may benefit from lower HbA1c targets ($\leq 6.5\%$) but the beneficial effects for DR progression are limited. For some patients, including those with cardiovascular disease or a record of severe hypoglycaemia, the risks in terms of mortality of a HbA1c target <7% may outweigh the benefits. A phenomenon observed in both the DCCT and UKPDS was rapid DR progression in a minority of subjects (13% in DCCT) within the first 18 months of the initiation of intensive therapy [143]. A similar observation was made in patients achieving excellent glycaemic control following combined renal and pancreas transplants [144]. Patients with poor glycaemic control, with diabetes of long duration and moderate

or severe non-proliferative DR require careful monitoring by an ophthalmologist for at least 12 months before and following initiation of intensive therapy.

Thiazolidinediones (glitazones) are hypoglycaemic agents used in type 2 diabetes. There is evidence of a beneficial effect of these agents on DR progression beyond their hypoglycaemic effects [145]. However, some studies have identified an increased risk of maculopathy with glitazone use [146]. A large prospective study of 170,000 subjects using the Diabetes Case Identification Database at Kaiser Permanente, Southern California, 9.9% of whom were taking glitazones, identified a 2.6-fold increased risk of developing diabetic maculopathy (95% CI 2.4–3.0) [147]. Other studies [145] including ACCORD [148] have not identified an increased risk of DMO. Current practice is to avoid glitazones in patients who develop maculopathy, particularly when peripheral oedema is also present.

3.5.3 Blood pressure

UKPDS

The UKPDS was the first study to demonstrate unequivocally that tight blood pressure control reduces both the macrovascular and microvascular complications of diabetes. 1148 subjects with type 2 diabetes and hypertension were randomised to either tight BP control with the angiotensin converting enzyme inhibitor (ACEI) captopril or the beta blocker atenolol as the main treatment (target <150/85 mmHg) or less tight control (target <180/105mmHg) [14]. After 8.4 years median follow-up the mean BP achieved in the 2 arms was 144/82mmHg and 154/87mmHg, respectively (a difference of 10mmHg systolic BP and 5mmHg diastolic BP). The tight control group demonstrated a reduction in risk of all diabetes related endpoints (24%; 95% CI 8%-38%; p=0.0046), deaths related to diabetes (32%; 6%-51%; p=0.019), and strokes (44%; 11-65%; p=0.013). There was a nonsignificant reduction in all-cause mortality.

The UKPDS showed a 37% reduction in risk of microvascular endpoints (95% CI 11%-56%; p=0.0092) mainly due to reduced risk of retinal laser treatment (Figure 3.2). At

9 years follow-up the tight control group demonstrated a 34% reduction in the rate of 2 step DR progression (99% CI 11-50%; $p = 0.0004$) and a 47% reduced risk of MVL (7-70%; $p = 0.004$). Reduction in MVL was primarily due to reduced incidence of maculopathy [149]. The authors of the UKPDS suggested that, as maculopathy generally responds less well to laser photocoagulation than PDR, reduced incidence of maculopathy was a particularly important clinical endpoint. While treatment of maculopathy has improved since the 1990s with the advent of intravitreal anti-VEGF agents this remains a valid claim.

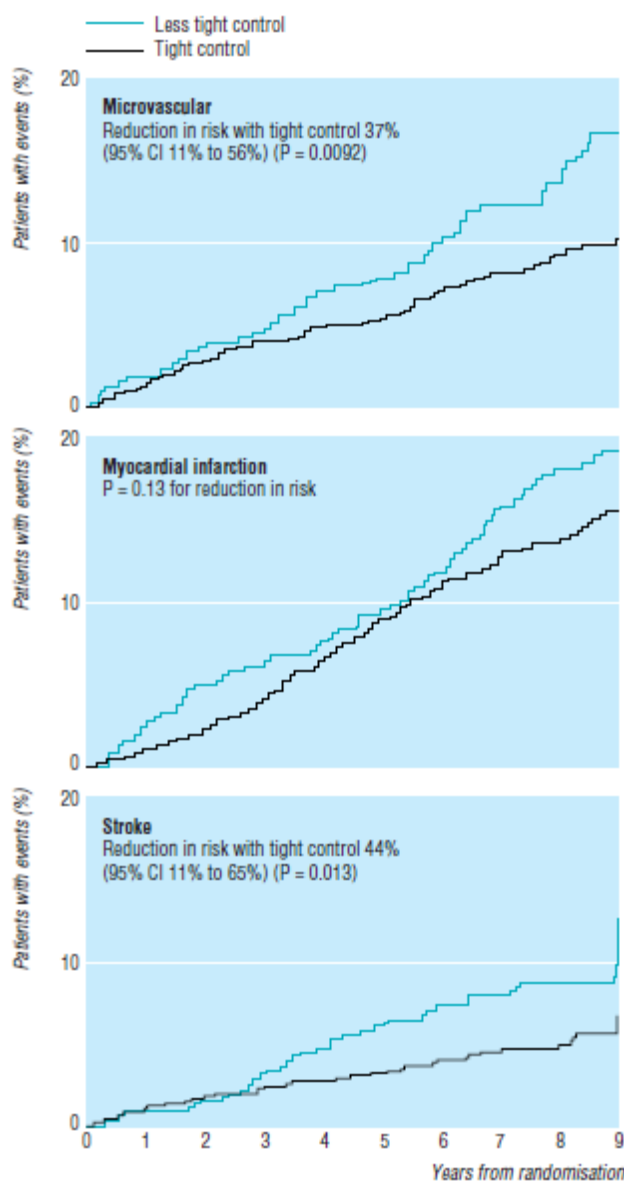


Figure 3.2 KaplanMeier plots of proportions of patients with tight or normal BP control who developed microvascular end points (mostly retinal photocoagulation), fatal or nonfatal myocardial infarction or sudden death, and fatal or nonfatal strokes in the UKPDS. Reproduced from [14].

Studies investigating more aggressive BP control

Results from the UKPDS, along with those from observational studies in type 1 diabetes [150,151], led to the introduction of guidelines for target blood pressure <130/80mmHg for persons with diabetes [152,153]. Subsequent studies investigated more aggressive BP control and lower thresholds for initiation of antihypertensive therapy. The ACCORD trial did not demonstrate a significant effect of intensive (targeting a sBP <120 mm Hg) versus standard (targeting a sBP <140 mm Hg) blood-pressure control on the progression of DR at 4 years (10.4% vs. 8.8%, $P = 0.29$)[136]. Similarly the ADVANCE study did not show a significant beneficial effect of intensive blood pressure control on progression of DR [140]. In this study a fixed combination of perindopril and indapamide or placebo were added to current antihypertensive therapy. The difference in sBP between the treatment groups was only 5.6 mm Hg, which may account for the lack of benefit seen. Interestingly, when all subjects in the trial were analysed together, maximum sBP and visit-to-visit variability in sBP were independent risk factors for both macrovascular and microvascular complications [151]. In the AdRem sub-study described above, in which 1241 subjects underwent photographic grading of DR, no benefit of more intensive BP control was shown in terms of DR incidence or progression. However, there was a significant reduction in incidence of maculopathy (OR 0.50, CI 0.29–0.88; $p=0.016$) in the intensive group [140].

In common with the above studies the Appropriate Blood Pressure Control in Diabetes (ABCD) randomised trial, showed no beneficial effect of intensive compared to moderate BP control [154]. Therefore trials since the UKPDS have failed to demonstrate a benefit in terms of DR incidence and progression with target blood pressures below those of the UKPDS. A possible explanation for these findings is the shorter follow-up of these more recent studies. An alternative or additional explanation is that the findings may demonstrate a ‘floor effect’ for the benefits of BP control. It is important to note that the evidence differs according to the starting level of blood pressure; it is more difficult for more recent trials to detect an effect.

Therapeutic issues in blood pressure control

Unresolved questions include whether particular anti-hypertensives confer benefit over others for persons with diabetes, whether particular agents provide advantages over and above their blood pressure lowering effects, and whether anti-hypertensive agents are beneficial for normotensive subjects with diabetes.

Components of the renin-angiotensin system are over expressed in the retina in diabetes [155]. A number of studies have investigated the effects of targeting this system with ACEIs or angiotensin 2 type 1 receptor (ATR-1) blockers. In addition to their effects on microvascular disease there is evidence of neuroprotection from these agents in DR in animal models [156]. The 'Steno 2' trial tested a multifactorial intervention including renin-angiotensin system blockers. As described above, subjects who received this intervention showed reduced risks of retinal photocoagulation as well as all-cause mortality, death from cardiovascular disease and progression to end-stage renal disease [141]. However, the trial design was not factorial and the effects of the individual elements of the intervention cannot be examined separately.

The EURODIAB Controlled Trial of Lisinopril in Insulin-Dependent Diabetes Mellitus (EUCLID) reported that in normotensive subjects (blood pressure $\leq 140/90$ mmHg), the ACE inhibitor lisinopril did not reduce the incidence of DR but decreased 2 step progression on the ETDRS scale and progression to PDR [157]. However, the placebo group in this trial demonstrated significantly higher HbA1c than the treatment group. When the results were adjusted for glycaemic control the difference in 2 step progression was no longer significant. Other limitations of this trial include its short follow-up (2 years) and the fact that it was underpowered to detect differences in DR which was not a primary outcome. In the Renin Angiotensin System Study (RASS) subjects with type 1 diabetes and normal blood pressure were randomised to enalapril (an ACE inhibitor) or losartan (an AT1-R blocker) or placebo. After 5 years, 2-step progression was significantly reduced in both the enalapril and losartan groups independent of changes in blood pressure or glycaemic control [158].

The Diabetic Retinopathy Candesartan Trials (DIRECT) programme was designed to address the question of whether the ATR-1 blocker candesartan could reduce incidence and progression of DR in subjects with type 1 and 2 diabetes [159,160]. Three randomised trials were conducted: a primary prevention study involving 1,241 subjects with type 1 diabetes and no DR (DIRECT prevent 1), a secondary prevention study of 1,905 subjects with type 1 diabetes and DR (DIRECT protect 1), and a secondary prevention study of 1,905 subjects with type 2 diabetes and DR (DIRECT protect 2). Subjects with type 1 diabetes were included if they were normoalbuminuric and normotensive (defined as BP \leq 130/85mmHg). Subjects with type 2 diabetes were included if they were normo-albuminuric and normotensive without anti-hypertensives or had BP \leq 160/90 on treatment. All subjects were randomised to candesartan 16-32mg day or placebo. Median follow-up was 4.7 years.

DIRECT-Prevent 1 showed a non-significant reduction (18% relative risk reduction; $p=0.051$) in incidence of DR. In a post hoc analysis, in which the primary end point was changed from 2 step to 3 step ETDRS progression, demonstrated a significant difference between treatment and placebo groups (35% RR reduction; $p=0.003$). DIRECT protect 1 did not show a difference between progression of DR between the treatment or placebo arms. In contrast, in type 2 subjects, DIRECT protect 2 reported a non-significant reduction in the progression of diabetic retinopathy (13% relative risk; $p=0.20$) and a significant 34% increase in diabetic retinopathy regression ($P=0.009$). Although DIRECT achieved none of its primary endpoints and there is no proof the beneficial effects observed were specific to blockage of the renin angiotensin system, this study suggests an overall beneficial effect of candesartan in DR.

The ADVANCE trial (described above) did not show a significant beneficial effect of intensive blood pressure control using a fixed combination of perindopril and indapamide on progression of DR [140]. When compared to DIRECT these results could suggest that candesartan but not ACE inhibitors might have beneficial effects in DR. However, ADVANCE showed a lower overall rate of DR progression limiting

power to detect moderate effects of the intervention. Therefore, while blood pressure control is a critical modifiable risk factor for the vascular complications of diabetes, questions remain over optimum management strategies.

3.5.4 Lipids

The first evidence of the role of lipids in the development of DR came in the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) [161]. Subsequently the ETDRS supported the link between lipid levels and development of maculopathy [162]. In the DCCT, increasing grade of DR was associated with raised triglycerides and negatively correlated with HDL cholesterol [163]. These findings, along with data to suggest that serum VEGF is raised in patients with hyperlipidaemia and reduced by lipid lowering agents [164,165], led to interventional trials of statins and fibrates in DR. In the Collaborative Atorvastatin Diabetes Study (CARDS) 2,838 subjects with type 2 diabetes were randomised to atorvastatin or placebo [166]. After median follow-up of 3.9 years a non-significant trend to reduced risk of laser photocoagulation was noted in the atorvastatin arm (OR 0.79; $p = 0.14$). There was no difference in progression of DR between the two groups. Progression of DR was not a primary endpoint in the CARDS and the trial was stopped 2 years earlier than planned because the pre-specified endpoint for efficacy in preventing acute coronary events and strokes had been met. However, this and other data suggests that the role of statins in DR is at best limited [167].

Fibrates are agonists for peroxisome proliferator-activated receptor- α (PPAR α) activation. Their pleiotropic actions include reduction in triglyceride levels, reduction in total and LDL cholesterol and increase in HDL cholesterol. PPAR α agonists are also reported to inhibit VEGF production and may have neuroprotective properties [168,169]. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study was a multinational RCT involving 9795 patients with type 2 diabetes aged 50-75 years and not taking statins [15]. Participants were randomised to fenofibrate 200mg daily or placebo. The need for laser photocoagulation was a pre-specified tertiary endpoint. In a sub-study 1012 subjects had retinal photography and grading of DR by ETDRS criteria.

Fenofibrate treatment was associated with reduced need for laser treatment (5.2% vs 3.6%, $p=0.0003$) in the whole study population. In the DR grading sub-study the number of events was small. The primary endpoint of the sub-study (2 step ETDRS progression) showed no difference between the two groups. However, fenofibrate was associated with reduced risk of 2 step progression in subjects who already had retinopathy ($p=0.004$). Subjects in the placebo arm had a higher rate of commencing statin therapy which may have masked a greater treatment benefit in this study. A further weakness of the study was the lack of predefined criteria for laser treatment. Interestingly the beneficial effects of fenofibrate observed in the FIELD study were independent of lipid levels, blood glucose control and blood pressure. Therefore the mechanism of action of fenofibrate in DR merits further study.

The ACCORD trial (described above) gave further evidence that fenofibrate could slow the progression of DR [136]. While intensive treatment of blood pressure in this trial did not show a significant reduction in DR progression, treatment of dyslipidaemia with 160mg fenofibrate plus simvastatin was effective compared to placebo plus simvastatin. Progression of DR by 3 or more ETDRS steps (or development of DR necessitating laser or vitrectomy) was reduced in the fenofibrate arm (6.5 vs 10.2%; adjusted OR: 0.60, 95% CI: 0.42–0.87; $p = 0.006$). The effect size was similar to that of intensive versus standard glycaemic control (7.3 vs 10.4%; adjusted OR: 0.67, 95% CI: 0.51–0.87; $p = 0.003$). In contrast to the FIELD study there was a difference in triglyceride levels at 1 year between the fenofibrate group (120 mg/dL) and the placebo group (147 mg/dL). This implies that the mechanism of effect may, in part, have been due to alterations in lipid levels. In summary, dyslipidaemia appears to play a role in the pathophysiology of diabetic retinopathy and maculopathy. However, the picture is complex and effective management strategies require further investigation.

3.5.5 HIV

Sub-Saharan Africa faces a growing problem of chronic non-communicable disease whilst concurrently experiencing continuing high rates of infectious disease. The interaction of infections such as HIV, TB and malaria with diabetes and its complications will be increasingly important as the prevalence of the disease grows. Persons with HIV are at increased risk of insulin resistance due to the pro-inflammatory effects of the virus, direct effects of antiretroviral therapy (ART) and indirectly through effects of ART on metabolism including body fat distribution. HIV lipodystrophy is a complex syndrome seen in people living with HIV for long duration most of whom are taking ART [170]. ART for HIV (specifically nucleoside reverse transcriptase inhibitors and protease inhibitors) has been associated with an increased risk of developing the metabolic syndrome [171].

Evidence of the effect of HIV on prevalence and incidence of microvascular complications of diabetes is extremely limited. Nephropathy is common in both diabetes and HIV. HIV associated nephropathy (HIVAN) is a complication of the infection which presents as proteinuria. American studies report that the condition principally affects subjects of African heritage [172,173]. Gupta et al reported that both diabetes and hypertension were associated with proteinuria in a large cohort of HIV positive subjects [174]. In 2007 a cross-sectional study of the complications of diabetes was performed at the diabetes clinic at Queen Elizabeth Central Hospital (QECH), Blantyre (described in detail in Chapter 4) [1]. In this study albuminuria was independently associated with duration of diabetes and HIV infection but not glycaemic control, blood pressure or age. Whether HIV and diabetes have a synergistic effect in the kidney and the mechanism of such an interaction is not known.

Distal predominantly sensory symmetrical polyneuropathy (DSSP) is a peripheral neuropathy attributable to the HIV infection itself or secondary effects of certain ART agents [175]. Diabetes has been associated with this condition [176,177]. However, differentiating peripheral neuropathies due to diabetes and HIV is likely to be difficult. The 2007 QECH study found no difference in prevalence of sensory

neuropathy between those subjects with and without HIV [1]. Very little is known about the interaction between HIV infection and DR. One case report suggested regression of DR following initiation of ART [178]. As part of the 2007 study at the QECH diabetes clinic our group reported retinal findings from 281 persons with diabetes. Prevalence of HIV in these subjects with diabetes was 13%; no association of STDR with HIV infection was found [2].

HIV has a number of manifestations in the posterior segment of the eye. HIV is associated with an increased risk of chorioretinal infection including viral retinitis (e.g. cytomegalovirus (CMV)), syphilitic retinitis and toxoplasma retinochoroiditis. Ocular and central nervous system non-Hodgkin's lymphoma occur more commonly in HIV. Approximately 75% of HIV infected persons develop microvascular abnormalities of the conjunctiva or retina. Retinal vessel tortuosity, cotton wool spots, telangiectasia, intra-retinal haemorrhages and venous and arterial occlusions are described. Proposed pathological mechanisms include HIV-induced thrombotic tendency, immune phenomena or as a direct result of HIV infection of vascular tissue [179].

3.5.6 Tuberculosis and malaria

A recent meta-analysis reported a 3-fold incident risk of tuberculosis in persons with diabetes [180]. The relative risk appears to be highest at younger ages [181]. It has been suggested that TB may increase the risk of diabetes and some TB treatments such as isoniazid have hyperglycaemic effects [182]. It is reported that concomitant disease is associated with worse outcomes for both diabetes and TB [182]. To my knowledge no high quality study has investigated the effects of TB on prevalence or progression of DR. Similarly little is known about the effects of malarial infection on the complications of diabetes. Repeated vascular endothelial activation in episodes of acute infection could predispose to endothelial perturbation and microvascular pathology. While diagnosis of acute infection is straight forward, quantifying the burden of malaria over a prolonged

epidemiological study is challenging. Therefore this is an extremely difficult topic to investigate.

3.5.7 Anaemia

Iron-deficiency and malaria-attributable anaemia are significant health problems in the developing world [183]. In Malawi the prevalence of anaemia in adult males and non-pregnant females is reported to be 32% and 71%, respectively [184]. The Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetic Study (SN-DREAMS) was a large Indian, population-based, cross-sectional study. 1414 subjects with diabetes were assessed for diabetes and systemic parameters. In a post hoc analysis of a subgroup of subjects with 'suboptimal' glycaemic or BP control ($\text{HbA1c} \geq 7\%$; $\text{BP} > 130/80$ mm Hg) factors associated with DR were presence of anaemia, younger age, male gender and microalbuminuria. A number of low-quality, cross sectional studies from Indian [185], Korea [186], China [187] and Iran [188] have also reported an association between low haemoglobin and DR.

3.5.8 Smoking

Smoking is an important determinant of the macrovascular complications of diabetes and is independently associated with progression of nephropathy in subjects with type 2 diabetes [189]. The WESDR found no significant relationship with incidence or progression of DR. However, smoking was associated with all-cause mortality [190]. In the UKPDS smoking was independently associated with reduced incidence of retinopathy and with reduced risk of DR progression [13]. A pharmacological effect of one of the components of tobacco smoke could explain this relationship. However, this would not justify the promotion of smoking in patients with diabetes because the deleterious effects of tobacco on cardiovascular and pulmonary health would far outweigh reduction in retinopathy risk in terms of overall morbidity.

3.6 Non-modifiable risk factors

3.6.1 Duration of diabetes

The Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) demonstrated that duration of diabetes is the most important non-modifiable determinant of development and progression of DR [83,84,190]. A potential confounder to the relationship of systemic parameters to DR is the period of undiagnosed disease in type 2 diabetes. A number of studies have estimated the delay between onset and diagnosis of type 2 diabetes using data on prevalence of DR. Using two distinct populations in Western Australia and Wisconsin, Harris *et al* reported a point estimate of the onset of detectable retinopathy of 4.2 to 6.5 years prior to diagnosis of diabetes [191]. The authors used very limited data based on a small number of subjects from the Whitehall cohort study [192] to estimate that the onset of diabetes itself could be approximately 5 years more (i.e. between 9 to 12 years in total).

In Scotland, Ellis *et al* graded retinopathy in 291 subjects with type 1 diabetes and 295 persons with type 2 diabetes and acquired data on duration of diabetes from a regional database [193]. Onset of detectable retinopathy was estimated to occur 5.7 years (95% CI 4.6 to 7.0) before diagnosis of diabetes. Using data on time to onset of STDR in type 1 diabetes this study estimated a 95% CI of length of preclinical diabetes to be between 3.0 and 9.4 years. Both the above studies used linear models which may be overly simplistic and both included stages of retinopathy not specific to diabetes. More recently Porta *et al* derived an estimate of 6.05 years between onset and diagnosis of type 2 diabetes using quadratic and linear models. These authors calculated appearance of 'moderate DR' 2.66 years before diagnosis of diabetes in patients with onset of diabetes ≥ 30 years of age. Moderate retinopathy was estimated to appear 3.29 years after diagnosis of diabetes in subjects with onset of diabetes < 30 years. These figures were added together to give an estimate of duration of diabetes before onset.

3.6.2 Type of diabetes

Diabetes can be classified into two groups: type 1 (previously known as ‘juvenile onset’) is associated with destruction of the pancreatic islet beta cells leading to failure of insulin production; type 2 (previously described as ‘adult onset’ or ‘non-insulin dependent’) diabetes is characterized by both insulin deficiency and insulin resistance. Epidemiological data on type 1 diabetes in Africa is sparse. Traditionally the proportion of type 1 diabetes was thought to be low although this could reflect high mortality [194]. Disease characteristics appear to differ from European populations. Peak age of onset is later in African communities, typically 22-29 years [194]. Other phenotypes of diabetes are recognised in patients of African origin. ‘Atypical African diabetes’ was first described in the 1960s. The most often reported atypical form is characterised by an initial clinical presentation of apparent type 1 diabetes with severe hyperglycaemia and ketosis, and subsequent long-term remission with or without relapses or a clinical course compatible with type 2 diabetes [195]. Malnutrition-related diabetes mellitus (previously known as ‘tropical diabetes’) is characterised by early-onset non-ketotic diabetes in underweight patients, with very high subsequent insulin requirements. Unlike true type 1 diabetes beta cell autoimmunity is not apparent [195].

Globally, the prevalence of DR differs between type 1 and type 2 diabetes. In a recent systematic review the age standardised prevalence in type 1 and type 2 diabetes of DR was 77.3% (95% CI, 76.3 – 78.3) and 25.2% (24.96– 25.36), respectively [196]. Figures for PDR, DMO and STDR were 32.4% (31.8–33.0) vs 3.0% (2.9 – 3.0), 14.3% (13.9–14.6) vs 5.6% (5.5–5.7) and 38.5% (37.8–39.2) vs 6.9% (6.8 – 7.0), respectively. These figures are not adjusted for other disease risk factors including duration of diabetes, glycaemic control and blood pressure which differ between type 1 and 2 diabetes. Studies reporting prevalence of DR are subject to classification issues: large studies and those in resource poor settings do not have access to the investigations necessary to reliably differentiate type 1 and 2 diabetes in all subjects.

3.6.3 Age

Features of sight threatening diabetic retinopathy (STDR) are generally not seen before puberty [197]. The reasons for this are unclear; levels of growth hormone, insulin-like growth factor and sex hormones such as testosterone and lower blood pressure have been suggested as potential explanations [198]. Most large epidemiological studies have found only very small effects of age on DR incidence and progression. There may be a tendency to reduced incidence of proliferative retinopathy in very old age. For example in the WESDR no subjects over 80 years developed PDR [197]. However, the numbers of subjects of this age in major studies are small.

3.6.4 Ethnicity

Clear differences in prevalence of diabetes between different ethnic groups have been reported. For example Pacific Islanders and Native Americans including the 'Pima Indians' have particularly high rates [199]. However, the literature on DR and ethnicity is much less clear. The Veterans Affairs Diabetes Trial (VADT) reported that the prevalence of DR equal to or greater than moderate NPDR differed significantly between Hispanics (36%), African Americans (29%) and non-Hispanic whites (22%) [200]. Differences were not accounted for by differences in age, duration of diagnosed diabetes, HbA1c or blood pressure. A UK study reported that patients of South Asian ethnicity demonstrated greater prevalence of DR and DMO than Caucasians [201]. However, these subjects also had worse blood pressure, HbA1c and total cholesterol as well as younger age at diagnosis. Socioeconomic status is an important, unmeasured potential confounder in the above studies. Interestingly, published rates of macrovascular complications in Africa are relatively low [194]. For example, prevalence of coronary heart disease in individuals with type 2 diabetes in sub-Saharan Africa is estimated at 5% [194]. Approximately half that in some European states [202]. The reasons for this remain unclear and the literature is by no means comprehensive.

3.6.5 Genetic factors

The genetics of DR is a large and rapidly evolving area beyond the scope of this thesis. An excellent recent review is available from Cho and Sobrin [203]. In brief, multiple genes are thought to influence DR phenotype. Twin and familial studies have demonstrated familial clustering [204]. Estimates of heritability for DR are as high as 27% and for PDR as high as 52% [205]. Linkage analyses, candidate gene association studies and genome-wide association studies (GWAS) have not yet identified reproducible loci for DR risk although associations with chromosomes 1, 3 and 12 have been reported [206]. The most studied individual genes are those encoding vascular endothelial growth factor, the receptor for advanced glycation end products and aldose reductase. GWAS methodology has been used successfully to study other complex polygenic diseases. Combination of large datasets from different ethnicities will be important in future to detect variants with sufficient effect sizes.

3.7 Chapter summary

In this chapter I have reviewed the major studies which have informed understanding of the determinants of severity and progression of DR. An excellent body of work exists concerning systemic risk factors such as glycaemic control, BP and lipids. The vast majority of studies were performed in Europe and North America. At present results are extrapolated to populations in Africa; primary evidence has yet to be accrued. Little is known about the effect on DR of a number of risk factors important to populations in Sub-Saharan Africa. These 'population specific risk factors' include HIV infection and anaemia. As the number of persons living with diabetes in Africa grows, understanding interactions between these diseases is becoming increasingly important for health service providers. Understanding epidemiological relationships is the first step to study of the pathophysiological interactions between disease states. In Chapter 4 of this thesis I will describe a systematic review of the available literature on the epidemiology of diabetic retinopathy and maculopathy in Africa. Having identified gaps in the

knowledge base of DR in Sub-Saharan Africa, the Malawi Diabetic Retinopathy Study will address some of these.

Chapter 4. Epidemiology of Diabetic Retinopathy and Maculopathy in Africa: a Systematic Review

4.1 Aims of chapter

This is the last section of my literature review. In this chapter I summarise findings from studies reporting the prevalence and incidence of diabetic retinopathy (DR) and diabetic maculopathy in African countries in light of the rising prevalence of diabetes mellitus. In the final part of the chapter I bring together findings from the literature review and present the hypothesis and aims of my thesis.

4.2 Introduction

The International Diabetes Federation (IDF) has estimated that the number of adults with diabetes in Africa will increase by 98%, from 12.1 million in 2010 to 23.9 million in 2030 [207] a presumed consequence of poor diet, sedentary lifestyles, obesity, population growth and ageing (in part due to successes in combating communicable diseases) [199]. 31 of the 48 least economically developed countries, as defined by the UN, are situated in Africa [208]. The epidemic rise in diabetes therefore poses significant public health and socioeconomic challenges for the continent.

As described in Chapter 1, diabetes causes visual impairment through cataract and DR, a progressive disease of the retinal microvasculature. DR can be graded on the basis of the clinical features as detailed in Chapter 2. The grades of retinopathy correlate with likelihood of development of proliferative DR and can be standardised by standard retinal photographs as used in the Early treatment of Diabetic Retinopathy Study (ETDRS) [19]. DR is the sixth leading cause of global blindness [8], and is one of nine target disease areas of the 'Vision 2020 action plan' a joint program of the WHO and the International Agency for the Prevention of Blindness. It is the leading cause of blindness in the working age population in the USA and in many European countries (and was until recently in the UK) [209]. With

increasing prevalence of diabetes in Africa, it is likely that DR will become an important cause of blindness. On this continent genetic factors, limited access to healthcare, and high rates of malnutrition, infectious disease, HIV and anaemia are likely to affect the spectrum of pathology encountered.

An overall assessment of current levels of DR in Africa has not been performed previously. In order to estimate the current and future burden of disease, to provide data to enable the assessment of changes that may result from service development, and to inform future research a systematic review of the epidemiological literature was required. The aim of this systematic review was therefore to summarise findings from reliable research studies of estimates of the prevalence and incidence of diabetic retinopathy and maculopathy in African countries. I completed this review under the supervision of Professor Paul Garner at Liverpool School of Tropical Medicine (LSTM); the results have been published [210].

4.3 Methods

4.3.1 Data sources and search strategy

I performed a systematic narrative review of published literature according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [211]. Relevant studies published between 1948 and February 2011 were identified by searching, using a pre-defined strategy, the following electronic databases: Medline (Pubmed), Embase (OVID) and Embase Classic, Science Citation index and Conference Proceedings Citation index (ISI Web of Science). The following were also searched: the African regional database 'African Index Medicus', the grey literature database 'OpenSIGLE', the WHO International Clinical Trials Registry and the meta-Register of Controlled Trials (mRCT). I developed customised searches with the help of Vittoria Lutje, a Cochrane Collaboration trained trials co-ordinator at the Cochrane Infectious Diseases group, LSTM. Search

histories are reproduced in Appendix 2. No language, publication status, time limits or language restrictions were applied to electronic searches. Search results were merged using reference management software (Endnote, Thomson Reuters) and duplicate records removed. The reference lists of articles identified, including existing reviews, were hand-searched.

4.3.2 Selection criteria

The following were included: studies reporting prevalence or incidence or progression of DR or diabetic maculopathy; cross sectional or cohort study design; studies of subjects with diabetes mellitus resident in African countries. Exclusion criteria were: studies with fewer than 50 subjects; studies of populations of African origin residing outside the continent; reports not published in English; case series and conference abstracts. To improve the current relevance of the review those reports published before 1990 were excluded.

The method used to apply selection criteria was as follows. I examined titles and abstracts and removed obviously irrelevant reports. I retrieved full text copies of the potentially relevant reports. Multiple reports of the same study were linked together. Full-text reports were examined independently by me and a colleague (Ian MacCormick, specialist trainee in ophthalmology and clinical PhD student at the Malawi-Liverpool-Wellcome clinical research programme) for compliance with eligibility criteria. Disagreements were resolved by discussion.

4.3.3 Data extraction and assessment of risk of bias

Major outcome variables were extracted independently by Ian MacCormick and me into a spreadsheet (Excel, Microsoft) with a standardised approach. The main outcome variables extracted were the prevalence of DR, proliferative diabetic retinopathy (PDR) and diabetic maculopathy and the incidence of DR, PDR and diabetic maculopathy. Prevalence of grades of retinopathy were recorded by patient according to the worse eye and, unless stated, are presented as such below. Studies were stratified by the source of the population sample (with community

studies more likely to give a more accurate population based assessment of prevalence); and risk of bias was assessed by seeking evidence of incomplete outcome data (missing data, subjects excluded from report, subjects lost to follow up in cohort studies).

4.4 Results

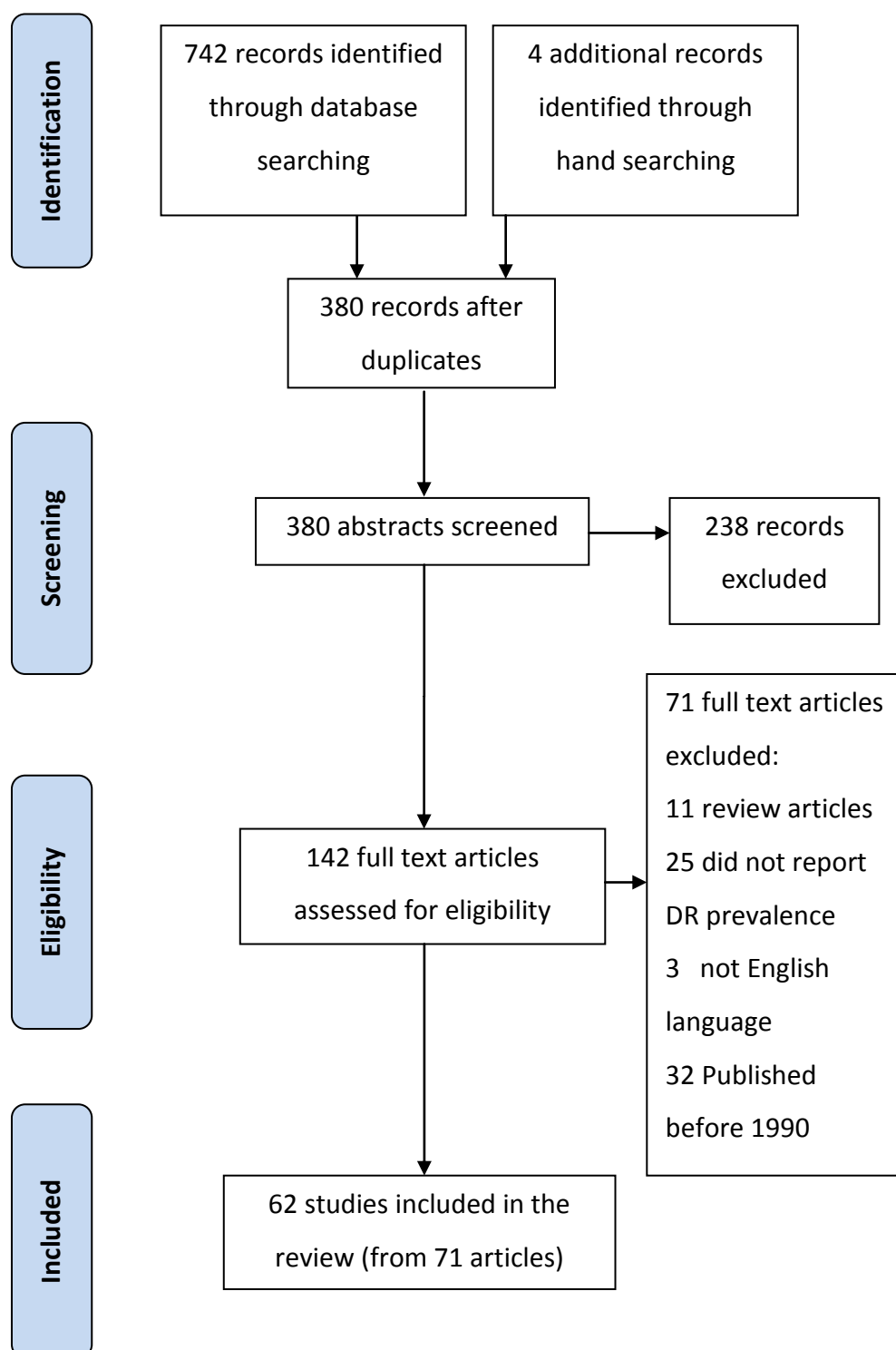
4.4.1 Literature search

The literature search yielded 380 citations of which 142 were reviewed in full text; 71 met the inclusion criteria and reported on a total of 62 studies (Figure 4.1) [2,212-281]. The literature search report is shown in Table 4.1.

Table 4.1 Literature search report for articles reporting prevalence, incidence or progression of DR or diabetic maculopathy in African countries. Four further articles were identified through hand searching and personal communication.

Source	Date range searched	Retrieved (before duplicate removal)
ELECTRONIC DATABASES		
Medline (Pubmed)	1948 – 6/2/2011	204
Embase (OVID) + Embase classic	1947 – 6/2/2011	333
Science citation index + Conference proceedings citation index (ISI web of science)	1900 – 7/2/2011	199
Total number of records in Endnote database after deleting duplicates		370
OTHER DATABASES		
African Index Medicus database	Searched 8/2/2011	5
OpenSigle	Searched 8/2/2011	0
ONGOING TRIALS REGISTERS		
WHO international clinical trials registry current controlled trials: meta register of controlled trials (mRCT)	Searched 8/2/2011	1

Figure 4.1 Identification process for eligible studies. Format reproduced from the PRISMA statement [6].



4.4.2 Characteristics of included studies

Characteristics of the included studies are summarised in Table 4.2.

Design: Only 3 community based studies were identified [212-214]. In Mauritius 1998 researchers followed up the population based study performed in 1992 [212] with a survey of the same cohort 6 years later [215]. An additional cohort study followed persons with type 1 diabetes identified from a hospital clinic [216-218]. All other studies were clinic-based surveys or case control studies; the majority were undertaken in diabetes clinics (hospital or primary care) or hospital ophthalmology clinics.

Distribution: The 62 studies were performed in 21 countries. Geographical distribution of studies was uneven and, within geographical regions, certain countries were over-represented. All of 19 studies undertaken in Western Africa took place in Nigeria except 1 which covered Nigeria and Ghana [219] and 1 from Mali [220]. Within East Africa 2 studies were conducted in the Seychelles [221,222] and 2 in Mauritius [212,215], relatively wealthy, ethnically diverse, small island nations. There was no clear correlation between the average standard of living in a country, as measured by per capita gross domestic product (GDP), and reported prevalence of DR (Figure 4.2) or PDR (Figure 4.3). Only five studies specifically reported data from rural populations [212-214,223,224].

Subject selection: Clinic-based studies were highly heterogeneous in patient selection in relation to age range, gender, ethnicity, duration and type of diabetes and co-morbidity. Of those studies conducted in diabetes clinics, 18 included all patients with diabetes attending the clinic while 14 confined their study to a subgroup, for example subjects with type 2 diabetes [225], children 5-18 years [226], or persons with duration of diabetes >5 years [227]. Of the 9 studies conducted in ophthalmology clinics 4 studied patients with a particular diagnosis (neovascular glaucoma [228], retinal disease [229,230], blind patients [231]), 1 studied persons attending specific diabetes eye clinics [222] and 4 studied a cross section of all eye patients [232-235]. In those studies that differentiated type 1 and

2 diabetes (30 studies) most used study specific definitions making inter-study comparisons problematic.

Assessment of retinopathy: Methods of assessment and classification of retinopathy varied widely. Only 9 studies used retinal photography [212,215,236-242] and 6 of these were conducted in South Africa [236-238,240-242]. 30 studies classified retinopathy simply as present or absent; 32 used a recognised grading system. Most used an adaptation of the ETDRS grading system [19]. However, the application and its reporting varied widely. In no study was an external validation of the practitioner's grading reported.

Evidence of bias: There was evidence of incomplete outcome data in a number of studies. In the majority of clinic based studies the number of subjects approached to participate was not reported making selection bias difficult to assess. Many studies reported prevalence of a number of diabetic complications. In some studies a low proportion of subjects were examined for retinopathy. For example, in Harzallah *et al* [243] only 19% of 593 subjects underwent retinal examination. Many studies excluded subjects with significant cornea or media opacities [244,245] or with ungradeable photographs [236].

Table 4.2 Characteristics of 62 studies reporting prevalence of diabetic retinopathy and maculopathy in Africa.

Characteristic	Geographical region					
	North Africa	Western Africa	Southern Africa	Middle Africa	Eastern Africa†	Total
Study design						
Community-based cross-sectional	1	1			1*	3
Cohort			1		1*	2
Diabetic clinic survey‡	4	5	10	2	11	32
Hospital eye clinic survey		5		1	3	9
Other hospital-based survey	2	5	2		2	11
Case control	2	3				5
Year published						
1990-1999	5	3	4	2	5	19
2000-2011	4	16	9	1	13	43
Type of diabetes						
Type 1 alone	1		2		1	4
Type 2 alone	4	8	1		2	15
Type 1 & 2 (separately)	1	2	3		5	11
Mixed	3	9	7	3	10	32
Practitioner grading retinopathy						
Ophthalmologist	1	12	7	3	11	34
Physician	4	3	2		2	11
Trained grader	1				2	3
Not specified	3	4	4		3	14
Instrument						
Slit lamp bio-microscopy	2	7	3	2	9	23
Retinal photo	1		6	1	2	10
Direct ophth'scope	1	5	4		1	11
Not specif'd/other	5	7			6	18
Other study characteristics						
Recognised grading system	4	5	10	2	11	32
Associations of DR reported	6	8	8	2	9	32
Vision reported	1	7	3	1	7	19
Total number of studies	9	19	13	3	18	

Geographical region defined according to UN scheme of geographical regions [282]. * Initial population based survey performed in Mauritius in 1992[212] followed up with a survey of the same cohort 6 years later [215]. † Includes 2 studies from the Seychelles and 1 from Mauritius. ‡ Hospital or primary care diabetes clinic.

Figure 4.2 Prevalence of DR in people with diabetes according to national per capita gross domestic product (GDP). Red markers: population-based studies. Blue markers: cohort and clinic-based studies. For cohort studies, prevalence in baseline survey shown. GDP per capita figures: IMF 2011 [283]. Abbreviations: USD United States Dollars.

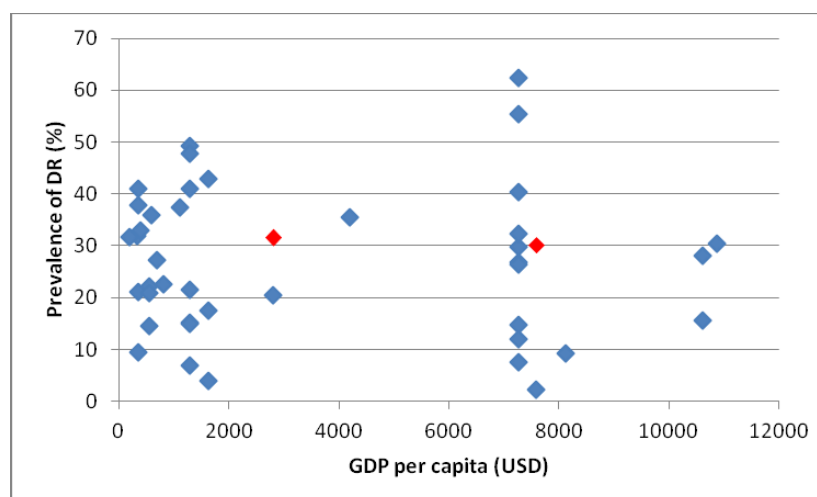
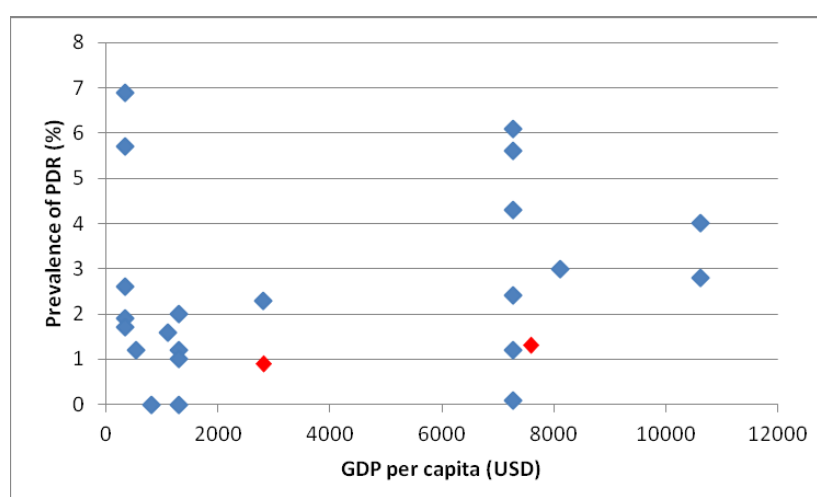


Figure 4.3 Prevalence of PDR in persons with diabetes according to national per capita gross domestic product (GDP). Red markers: population-based studies. Blue markers: cohort and clinic-based studies. For cohort studies, prevalence in baseline survey shown.



4.4.3 Community based studies

I identified 3 community-based studies (Table 4.3). In Egypt 1991-4, researchers examined the prevalence of diabetes and the relationship between HbA1c and retinopathy [213,239,246,247]. Articles by Herman [239] and Penman [246] report different prevalence of DR as graded from retinal photography: 35.4 % in 376 subjects in the former and 31.6% in 335 subjects in the latter. No explanation for this difference between the two reports is offered suggesting missing data in one or both analyses. Herman *et al* [239] demonstrated in multivariate analysis that DR was associated with longer duration of diabetes (per 10 years) (OR = 1.37, 95 % CI 1.09, 1.73) and higher HbA1c (per unit) (OR = 1.15, 95 % CI 1.03, 1.27).

Researchers in Mauritius 1992 [212] investigated prevalence of and risk factors for DR in Asian, Indian, Chinese and Creole Mauritians. This high quality study demonstrated a high prevalence of DR in all major ethnic groups in Mauritius. The prevalence of DR and PDR were particularly high in known diabetes: 44.3% and 2.3%, respectively. Muslim Indians had the lowest prevalence of DR: 10.8% and 34.0% for new and known diabetes, respectively); significantly lower than Creoles (18.8% and 53.8%, respectively). The following were independently associated with retinopathy: duration of diabetes, fasting plasma glucose, systolic blood pressure, albuminuria and decreasing body mass index.

4.4.4 Cohort studies

Table 4.4 summarises cohort studies of DR conducted in Africa. In Mauritius 1998 researchers followed up the population based study performed in 1992 [212] with a survey of diabetes complications [215]. Of subjects with diabetes in the initial survey 40.5% were re-examined. The 6 year incidence of DR and PDR in subjects with diabetes but no DR in the first survey was 23.8% (95% confidence interval 18.3-29.3) and 0.4% (0-1.2), respectively. The incidence of PDR was much higher in subjects with mild non-proliferative diabetic retinopathy (NPDR) (5.2%) and moderate NPDR (29.4%) in the first survey. Duration of diabetes and fasting blood glucose were independently associated with incidence of retinopathy. In South Africa 1982-2002 Gill and co-workers identified a cohort of patients with diabetes

diagnosed before age 30 years requiring insulin therapy [216]. In those subjects seen at 10 years prevalence of DR had increased from 6% to 52% and PDR from 0 to 3% [217]. In subjects seen at 20 years prevalence of DR had increased from 12% to 59% [218]. Incidence of retinopathy was not reported in these studies.

No other prospective cohort studies were identified. However, studies reflecting cumulative incidence of DR are available. In South Africa, Distiller et al [237] reported on 1520 type 1 and 8026 type 2 patients who had maintained membership for ≥ 5 years of a community-based, privately funded diabetes management program. In type 1 subjects prevalence of any retinopathy at baseline and at 5 years was 22.3% and 28%, respectively and in type 2 subjects 20.5% and 26.6%, respectively. In retrospective studies of persons with diabetes of long duration Lester [248] showed a prevalence of DR of 45.5% in 121 Ethiopian patients with duration of diabetes > 20 years while Distiller [238] reported presence of DR in 14.8% of 148 South African Caucasian subjects with type 1 diabetes of >18 years duration.

Table 4.3 Community based cross-sectional studies reporting prevalence of DR in Africa

Study	Methods	Subjects and sub-groups	n	Outcome		
				Any DR % (95% CI)	PDR (%)	Mac. (%)
Studies reporting prevalence of diabetes and DR in the general population						
Egypt, 1991-4 [213, 239, 246, 247]	Stratified random sampling of persons ≥20 years in urban and rural areas near Cairo. 4620 adults underwent random glucose testing. Those at high risk of diabetes and a sample of those at low risk (total 1451) had fasting glucose test. Diabetes diagnosed by WHO criteria [online supplementary reference 101]. RP graded according to Airlie House Classification and BIO exam by ophthalmologist. Those ungradeable on photography and BIO excluded from analysis of RP and BIO, respectively.	Subjects with diabetes (RP)*	335	31.6	0.9	4.5‡
		Subjects with diabetes (BIO)*	404	20.3	0	1.2‡
		Known diabetes (RP) §	287	41.5 (35.8-47.2)		
		Newly diag diabetes (RP)§	89	15.7 (8.2-23.3)		
		Impaired GT (RP)§	103	1.9 (0-4.6)		
Mauritius 1992 [212]	6553 persons in 14 geographically defined clusters underwent GTT. In 11 clusters all adults aged 25-74 were invited to attend; in 3 clusters age stratified sampling of adults 35-64 performed. Those with diabetes and 25% of those with impaired GTT (WHO criteria(115)) had 3-field, 45° stereoscopic RP of the right eye. Grading by certified assessor according to modified Airlie House criteria. Those with ungradeable photographs excluded.	All subjects with diabetes	746	30.2 (26.9-33.5)	1.3	
		Known diabetes	388	44.3 (39.4-49.2)	2.3	
		Newly diagnos'd diabetes	358	14.8 (11.1-18.5)	0.3	
		Impaired GT	165	9.1 (4.7-13.5)	0	
		Indian race with diabetes	186	22.8 (18.7-28.9)	1.1	
		Creole race with diabetes	160	35.7 (28.1-43.3)	1.3	
Study of cause of visual impairment in the general population						
Nigeria, 2005-2007 [214]	National multistage, stratified cluster sampling of persons ≥40 year to determine cause of VI. 13591 VA tested; 3129 had uncorrected VA <6/12 in better eye examined by ophthalmologist. Primary cause of VI recorded.	Subjects with VA <6/12 better eye	3129	0.29		

The Prevalence of DR in Egypt 1991-4 was reported in four publications: * denotes data from Penman 1998 [246], § denotes data from Herman 1998 [239]. ‡ Maculopathy in Penman 1998 [246] defined as any exudates present in macular region. Abbreviations: RP retinal photography; BIO binocular indirect ophthalmoscopy; DO direct ophthalmoscopy; GTT Glucose tolerance test; VA visual acuity; VI Visual impairment.

Table 4.4 Cohort studies reporting prevalence and incidence of DR in Africa.

Study	Methods	Subjects and sub-groups	n	Outcome		
				Any DR % (95%CI)	PDR %	Progres sion (%)
1. Mauritius 1992-1998						
Initial population based study 1992 [212]	Population based study of prevalence of diabetes and DR: methodology outlined in Table 1	All subjects with diabetes	746	30.2 (26.9-33.5)	1.3	
		Known diabetes	388	44.3 (39.4-49.2)	2.3	
		Newly diagnosed diabetes	358	14.8 (11.1-18.5)	0.3	
Second survey 1998 [215]	Of those assessed for complications in 1992, 528 attended the follow-up survey. Grading of retinopathy as in first assessment.	Subjects with diabetes	302	33.8	3.0	25.2
		Diabetes with no DR at baseline	227	23.8*	0.4†	23.8
		Diabetes, mild NPDR at baseline	58		5.2†	27.7
		Diabetes, mod. NPDR at baseline	17		29.4 †	35.3
2. South Africa 1982-2002						
Baseline assessment 1982 [216]	88 black South Africans with diabetes requiring insulin therapy diagnosed <30yrs attending the diabetes clinic at Baragwanath Hospital, Soweto screened for diabetic complications. 66 examined by a physician using DO.	Subjects with diabetes requiring insulin therapy diagnosed <30yrs	66	12.1	0	
		Sub-group seen at 10yrs	33	6	0	
		Sub-group seen at 20 yrs	17	12		
10 yr follow-up 1992 [217]	Of the original cohort 24 were lost to follow-up, 10 had died. Of 54 still attending clinic 36 were examined. In 3 patients cataracts prevented fundal view.	Subjects with diabetes requiring insulin therapy diagnosed <30yrs	33	52	3	
20 yr 2002 [218]	Of the original cohort 21 died, 39 lost to follow-up, 28 still attending clinic, of which 17 were assessed.	Subjects with diabetes requiring insulin therapy diagnosed <30yrs	17	59		

* Incidence of DR at 6 years † Incidence of PDR at 6 years. PDR Proliferative diabetic retinopathy; NPDR Non-proliferative diabetic retinopathy.

4.4.5 Hospital-based and primary care-based surveys

Tables 4.5, 4.6 and 4.7 summarise hospital based and primary care based surveys reporting prevalence of DR using a recognised grading system. The most recent large study from Northern Africa was conducted in Cairo during 2007-2008 in endocrinology clinics in 2 major teaching hospitals [244]. Prevalence of PDR (2.3%) and clinically significant macular oedema (CSMO) (11.5%) reported in this study were high. Of four studies from Western Africa [249-252] none reported the prevalence of maculopathy (Table 4.5). Only three studies were identified from Middle Africa [227,253,254]. Longo-Mbenza *et al* [253] studied 3010 persons with diabetes attending diabetes primary care facilities using retinal photography; prevalence of DR was 31.6%.

Hospital based surveys from Eastern Africa cover 9 countries showing a general trend of increasing prevalence of DR from earlier to more recent studies (Table 4.6). Diabetes clinic based surveys from Southern Africa in general report higher prevalence of DR and PDR than comparable clinics in other regions of Africa (Table 4.7). PDR prevalence greater than 4% was recorded in 3 studies of unselected diabetes clinic attendees from South Africa [224,241,255]. Data on prevalence of diabetic maculopathy was limited from all regions. However, 8 studies suggest high prevalence [2,222,224,230,241,244,255,256,]. Of note, 3 South African, primary care-based studies were identified. Levitt [255], Mash [241] and Read [256] reported high prevalence of PDR and maculopathy (Table 4.7), comparable to hospital based surveys in the same country and higher than hospital based surveys elsewhere in Africa.

Two studies from South Africa compared prevalence of DR in different ethnic groups [240,256]. The authors acknowledge the effect of environmental factors on different racial communities even in the post-apartheid era. Kalk *et al* [240] studied 507 'poor or indigent' persons attending a free hospital diabetes clinic. Prevalence of DR was similar in persons of African (37%), European (41%) or Indian (37%) heritage. However, 'severe DR' (study specific classification) was significantly more

frequent in Africans (52%) and Indians (41%) compared to Europeans (26%). Read *et al* [256] found no relationship between ethnicity and DR prevalence.

Table 4.5 Hospital based surveys of persons with diabetes reporting prevalence of DR using a recognised grading system in Northern, Western and Middle Africa.

Study	Country	Methods	n	Any DR (%)	PDR (%)	CSMO (%)
Notthern Africa						
Elbagir 1995 [257]	Sudan	Persons with diabetes requiring insulin (duration >1 yr) aged 15-75yrs attending medical OPD examined with DO by a physician	91	43	10*	NR
Macky 2011 [244]	Egypt	Pts >18yrs age attending a diabetes clinic examined with SL by Ophthalmologist. Excluded 47 pts due to media opacities	1325	20.5	2.3	11.5
Western Africa						
Ikem 2001 [252]	Nigeria	Consecutive subjects with type 2 diabetes seen at medical OPD. Examined by physician; instrument not stated.	132	41.1	1.0	NR
Alebiosu 2003 [249]	Nigeria	Hospitalised subjects with type 2 diabetes and nephropathy. Examined with DO by physician.	191	47.1	12.6	NR
Omolase 2010 [250]	Nigeria	Persons with diabetes attending medical OPD. Examined with DO by Ophthalmologist.	100	15.0	2.0	NR
Onakpoya 2010 [251]	Nigeria	Type 2 patients attending a 3° centre diabetes clinic; invited for screening by Ophthalmologist with DO. 3.6% NFV.	80	21.6	1.2	NR
Middle Africa						
Sobngwi 1999 [254]	Came-roon	Adults attending diabetes clinic. Excluded pts with renal disease. SL exam by Ophthalmologist.	64	37.5	1.6	NR

* 'Severe retinopathy' by WHO multinational study criteria [284]. Abbreviations: Pts Patients; IO Indirect ophthalmoscope; DO Direct ophthalmoscope; SL Slit lamp biomicroscopy; RP Retinal photography; OPD Out-patient department; NFV No fundal view; DRC Democratic republic of Congo; NR Not reported.

Table 4.6 Hospital based surveys of persons with diabetes reporting prevalence of DR using a recognised grading system in Eastern Africa

Study	Country	Methods	n	Any DR (%)	PD R (%)	Any maculopathy (%)
Sullivan 1990 [221]	Seychelles	Persons with diabetes requiring insulin therapy attending diabetic clinic examined by a physician. Instrument NR.	108	15.7	2.8	NR
Lester 1992 §	Ethiopia	Type 1 diabetes seen 1976-90. Exam by physician. Instrument NR.	431	9.5	2.6	1.2
Lester 1993 §	Ethiopia	Type 2 diabetes seen 1976-91. Exam by physician. Instrument NR.	503	41.1	6.9	4.0
Taylor 1997 [222]	Seychelles	Type 2 diabetes: 184 attending an eye clinic, 199 invited for screening. Ophthalmologist SL exam	383	28	4	19
Seyoum 2001 [258]	Ethiopia	Persons attending a diabetes clinic. DO exam by Ophthalmologist. 3 subjects excluded as NFV.	302	37.8	1.7	NR
Teshome 2004 [230]	Ethiopia	Consecutive patients seen at a retinal clinic (not all had diabetes). SL exam by Ophthalmologist.	1390	28.7	9.9	11.1‡
Mumba 2007 [259]	Tanzania	Pts >18yrs attending diabetes clinic. No previous fundus exam. SL exam by Ophthalmologist	86	20.9	1.2	NR
Mwale 2007 [245]	Kenya	Type 2 diabetes clinic pts. SL exam by Ophthalmologist. Excluded cornea or media opacity.	96	22.6	0	NR
Gill 2008 [223]	Ethiopia	Consecutive pts attending hospital diabetes clinic in a remote region. SL exam by Ophthalmologist.	105	21	1.9	NR
Glover 2011 [2]	Malawi	Consecutive adults attending a hospital diabetes clinic. SL exam by Ophthalmologist.	281	32.0	5.7	15.0*

* Sight threatening maculopathy according to Liverpool Diabetic Eye Study adaptation of the Early Treatment Diabetic Retinopathy Study (ETDRS) grading [285]. ‡ Clinically significant macular oedema. § Multiple publications [248,260,261] with overlapping populations have emanated from the diabetes clinic at Yekatit 12 Hospital, Addis Ababa, Ethiopia. For the purposes of this review these papers are viewed as one study. Data presented from Lester 1992 [260] and Lester 1993 [261]. Abbreviations as Table 4.

Table 4.7 Hospital-based and primary care-based surveys of persons with diabetes reporting prevalence of DR using a recognised grading system in Southern Africa

Study	Country	Methods	n	Any DR (%)	PDR (%)	CSMO (%)
Mollentz 1990 [242]	South Africa	Black subjects with diabetes >5yrs duration attending diabetes clinic. RP graded by Ophthalmologist.	86	29.7 ‡	1.2‡	NR
Levitt 1997 [255]	South Africa	Black Africans attending diabetes primary care service. Examined by a physician with DO	243	55.4	4.3	31.1†
Rotchford 2002 [244]	South Africa	Adults attending a nurse-led primary care diabetes service in rural KwaZulu-Natal. Examined with SL by an Ophthalmologist.	253	40.3	5.6	10.3
Huddle 2005 [262]	South Africa	Pregnant women with diabetes attending a clinic: type 1, type 2 and gestational diabetes. DO exam; practitioner grading retinopathy NR.	733	7.6	0.1	NR
Carmichael 2005* [236]	South Africa	Persons attending an urban diabetes clinic: 588 black, 739 white, 180 indian. RP graded by Ophthalmologist. Ungradeable photographs excluded.	1517	26.5	NR	NR
Mengesh 2006 [263]	Botswana	Persons with diabetes attending government health facilities. SL exam by Ophthalmologist.	401	9.2	3.0	NR
Mash 2007 [241]	South Africa	Persons attending primary care diabetes service: 44% 'black'; 56% 'coloured'. RP graded by Ophthalmologist. 17.5% photographs ungradeable.	400	62.4	6.1	15.2†
Read 2007 [256]	South Africa	Type 2 pts attending a primary care diabetes clinic (124 'Black'; 119 'Coloured'; 5 'White'; 1 'Asian'). DO exam by Ophthalmologist.	248	32.3	2.4	8.5

*Three reports [236,240,264] described grades of DR in overlapping populations. Figure for any DR taken from the largest report [236] (n=1517). † Any maculopathy. ‡Percentage of eyes (not patients) with specified grade of DR. Abbreviations as Table 4.

4.4.6 Studies reporting visual acuity

Nineteen studies reported visual acuity (VA) in subjects with diabetes; parameters reported varied widely between studies. Only the Nigerian national blindness and visual impairment survey [214] tested logMAR acuity. The population-based Mauritius diabetes complication study [212] reported best correct visual acuity (BCVA) <6/12 in 7.1% of subjects with diabetes at baseline. There was no difference in this figure for subjects with and without retinopathy. The Diabetes in Egypt project [246] reported VA in 427 subjects with diabetes. Of these 31 (7.3 %) were blind (defined as BCVA in the better eye less than 6/60); 239 (56 %) had a BCVA between 6/12 and 6/60. It is likely that media opacities accounted for a proportion of this visual impairment: 123 eyes had cataract; 11 had corneal opacity; 17 had both.

The Nigerian national blindness and visual impairment survey was conducted between 2005 and 2007 [214]. DR was identified as the primary cause of visual impairment in 0.29% of 3129 subjects with uncorrected VA worse than 6/12 and in 0.5% of those with acuity less than 3/60. This study is likely to underestimate the visual impact of DR as examiners were instructed to preferentially record treatable, then preventable causes of visual impairment i.e. cataract would be recorded in preference to DR if both were affecting visual acuity to similar degrees.

4.5 Discussion

4.5.1 Overall commentary

This systematic narrative review describes 62 studies reporting the prevalence and incidence of DR and maculopathy in Africa before February 2011. The methodological approach used standard inclusion, appraisal and data extraction techniques. Few high-quality, population-based studies were identified; the majority of studies were surveys of hospital clinic attendees. Identified studies were highly heterogeneous in terms of subject selection and method of assessment

and classification of retinopathy. Despite these inconsistencies between studies, the review identified rates of DR prevalence in many areas of Africa comparable with high-income countries. Prevalence of PDR and maculopathy was high in recent studies particularly those from Southern and Eastern Africa. Common themes were identified in the associations of DR and impact on vision.

4.5.2 Methodology of included studies

The review identified 3 high quality, population-based, cross-sectional studies of DR epidemiology [212,213,214]. Only two cohort studies were identified. Large epidemiological studies are expensive; the population-based studies were conducted in states with relatively greater resources: Nigeria, Mauritius and Egypt. The lack of studies from Middle Africa is likely to reflect lack of resources, poor health infrastructure, and deficiency of trained medical professionals. The relatively small number of studies identified from Northern Africa is partially explained by the tendency of francophone countries to publish in French.

The literature is dominated by studies of urban populations reflecting the distribution of major health facilities. Urbanisation is seen as an important factor driving the diabetes epidemic [286]; studies including only urban populations may over-estimate overall prevalence of DR. A caveat is that in resource poor settings patients travel long distances to health facilities and rural patients may therefore be included. The majority of studies identified were hospital clinic-based surveys; selection bias is a major issue and the findings should be generalised to other settings with caution. Another bias is that clinics are seen by many as a point to collect medication; persons with diet-controlled diabetes may be under-represented. The classification of diabetes in Africa is problematic particularly where investigations are limited. Disease characteristics differ from Caucasian populations. For example, peak age of onset of type 1 diabetes is later in African communities, typically 22-29 years [194]. Other phenotypes of diabetes are recognized in people of African origin including 'atypical African diabetes' and 'malnutrition-related diabetes' [195].

Adaptations of the ETDRS grading system have become the accepted reference standard for classifying retinopathy in research settings. Despite this, its use in everyday clinical practice is difficult due to a large number of levels requiring correlations with standard photographs and grading rules which must be remembered. General ophthalmologists and physicians in resource poor settings may not be able to use this system to a reproducible level. Stereoscopic photography with validated grading is the reference standard for assessing retinopathy. Digital photography allows transfer of images to distant reading centres as performed in the Diabetes in Egypt project [213,239,246,247]. This will be one direction of future research.

4.5.3 Prevalence and incidence of DR and diabetic maculopathy

Community-based studies identified in this review reported prevalence rates of DR and PDR comparable with American and European diabetic populations. The Diabetes in Egypt project [213,239,246,247] reported a prevalence of DR and PDR in subjects with diabetes of 31.6% and 0.9%, respectively. The Mauritius diabetes complication study [212] reported 30.2% DR and 1.3% PDR; the prevalence of PDR in subjects with known diabetes was 2.3%. In comparison, a 2005-2008 cross-sectional sample of US adults with diabetes aged 40 years and older estimated prevalence of DR and PDR as 28.5% and 1.5%, respectively [287]. Recent population based studies in Europe have reported similar rates [102,288-291]. Younis *et al* [288] studied 8062 persons with diabetes entering an English primary care-based screening program. The prevalence of any retinopathy and PDR in type 1 diabetes was 45.7% and 3.7%, respectively, and in type 2 diabetes 25.3% and 0.5%, respectively.

The lack of community-based studies from Sub-Saharan Africa is important. Very high prevalence of DR, PDR and maculopathy has been reported in notable high-quality, clinic-based surveys in the last decade: in Eastern Africa by Glover *et al* [2] (32.0% DR, 5.7% PDR, 15% sight threatening maculopathy), and in South Africa by

Mash [36] (62.4% DR, 6.1% PDR, 15.2% any maculopathy) and Rotchford [224] (DR 40.3%, PDR 5.6%, 10.3% CSMO). These figures are likely to reflect factors including ethnicity, poor access to medical services, late diagnosis, and co-pathology including infection (importantly HIV and malaria), hypertension, malnutrition, and anemia. We found no clear relationship between per capita GDP and prevalence of DR or PDR. However, the increased infrastructure to detect disease in states with greater resources is an important confounding factor.

The influence of ethnicity on DR prevalence in populations of African origin has yet to be determined. In the USA, Zhang *et al* [287] reported prevalence of both DR and vision threatening retinopathy (defined as ETDRS severe non proliferative diabetic retinopathy, PDR, or CSMO) to be higher in non-Hispanic black subjects (38.8% and 9.3%, respectively) compared to non-Hispanic whites (26.4% and 3.2%, respectively). Previous studies have shown similar results [292,293]. However, differences were attributable to risk factors for retinopathy [293]. Therefore no ethnic propensity to retinopathy has been identified [294].

Neither of the two cohort studies identified by this review reported 2 or 3 step progression on the ETDRS scale as used in recent European studies [136]. The Mauritius diabetes complication study [215] reported 6-year incidence of DR (23.8%). Six-year progression to PDR was reported from no DR (0.4%), mild NPDR (5.2%) and moderate NPDR (29.4%). The United Kingdom Prospective Diabetes Study (UKPDS) reported similar 6-year incidence of DR: 22% [13]. However, the UKPDS population were studied from a later time point: clinical diagnosis of diabetes. In the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) 4-year progression of DR and progression to PDR was observed in 41.2% and 10.5% of type I subjects, 34% and 7.4% of insulin-treated type II subjects and 24.9% and 2.3% of non-insulin treated subjects, respectively [295].

4.5.4 Impact of diabetic retinopathy on vision

Estimates of the proportion of African persons with diabetes who are visually impaired are high even compared to older European and American studies. 7.3 % of subjects in the Diabetes in Egypt project [246] had BCVA in the better eye less than 6/60. In contrast, of the WESDR population 3.6% of subjects aged less than 30 years at diagnosis, and 1.6% of subjects aged greater than or equal to 30 years at diagnosis were legally blind according to US standards [10,11]. The WHO estimates that in the United States and Canada 17% of blindness is attributable to DR [296]. While data is sparse, the proportion of visual impairment and blindness due to DR in Africa appears to be considerably less. However the prevalence of visual impairment and blindness is significantly higher in Africa [296] reflecting high prevalence of pathologies including uncorrected refractive error, cataract, corneal opacities and glaucoma.

4.6 Conclusions of systematic review

The findings of this review have important implications for both research and clinical practice and can be summarised thus:

- There is a paucity of high quality epidemiological data on DR in Africa
- Existing estimates of prevalence of any DR, proliferative DR and maculopathy are comparable to recent European and American studies
- Large, community-based cross-sectional and cohort studies are needed to investigate rates and determinants of DR prevalence, incidence and progression in Africa
- Consensus is needed on the most appropriate methods of identification and classification of retinopathy for research and clinical practice

4.7 Summary of the literature review; aims and objectives of thesis

4.7.1 Findings of the literature review: Chapters 1, 2, 3 and 4

The pathophysiology of DR is complex. Dysfunction of the vascular endothelium is an important component and can be assessed by measurement of serum biomarkers and *in vivo* functional testing. The clinical features of DR are well described. There are many grading schemes for DR. The reference standard is the ETDRS scale: levels are validated for progression of retinopathy and visual loss as described in the WESDR and the ETDRS. Other grading schemes including the LDES scale map to ETDRS levels providing an evidence base for treatment thresholds and facilitating studies of the determinants of severity and progression of DR.

An solid body of work exists concerning systemic risk factors such as glycaemic control, BP and lipids. The vast majority of studies were performed in Europe and North America. At present results are extrapolated to populations in Africa; primary evidence has yet to be accrued. Little is known about the effect on DR of a number of risk factors important to populations in Sub-Saharan Africa. These ‘population specific risk factors’ include HIV infection and anaemia. There is a paucity of high quality epidemiological data on DR in Africa. Large, community-based cross-sectional and cohort studies are needed to investigate rates and determinants of DR prevalence, incidence and progression in Africa.

4.7.2 Previous work on diabetes complications at Queen Elizabeth

Central Hospital

In 2007 a cross-sectional study of the complications of diabetes was performed at Queen Elizabeth Central Hospital (QECH), Blantyre [1]. 620 consecutive patients attending the diabetes clinic were assessed. Subjects completed a questionnaire and were examined for BP, BMI and visual acuity and underwent a neurovascular assessment of the feet. Fasting plasma glucose (FPG), HbA1c and HIV tests were performed. A subgroup of subjects had urine albuminuria and serum creatinine

measured. In this study control of glycaemia and hypertension were poor: mean FPG 182.7 mg/dl (SD 92, range 11.6--580); mean HbA1c 9.4% (SD 2.5, range 5--19.6); 52% of subjects had sBP \geq 140 mmHg. 13.7% of subjects were HIV positive. Microvascular complication rates were high. Prevalence of nephropathy (defined as \geq 1+ albuminuria on dipstick testing) was 34.7%. HIV positive subjects were more likely to have albuminuria (48.0% v 33.3% $p<0.05$). Objective evidence of neuropathy was present in 33.1%.

A subgroup of 281 subjects from the cohort were examined by one ophthalmologist from our group [2]. Sampling was ad hoc: subjects were examined when the ophthalmologist was present. Retinopathy was assessed by clinical ocular examination at the slit lamp and graded according to the LDES scale. Prevalence of any DR, STDR and PDR in type 1 diabetes was 28.1% (95% CI 12.5 to 43.7%), 18.8% (5.2 to 32.2%) and 12.5% (1.0 to 24.0%), respectively. In type 2 diabetes prevalence of any DR, STDR and PDR was 32.5% (26.7 to 38.3%), 19.7% (14.7 to 24.6%) and 4.8% (2.2 to 7.5%), respectively. In multivariate analysis STDR was associated with albuminuria (OR 2.6; $p=0.02$), neuropathy (OR 3.4; $p=0.005$) and insulin use (OR 5.3; $p=0.0004$), but not with HIV status. Strengths of this study included its relatively large sample size, use of a detailed grading system for DR and collection of data on HIV status. Principle limitations of the work were that this was a single centre, clinic-based study, the lack of systemic sampling and absence of external validation of retinopathy grading.

4.7.3 Hypothesis

In the knowledge of the important data from QECH described above, I planned to investigate further the epidemiology of DR in Southern Malawi. Studies presented in this thesis are based on the hypothesis that, in the Malawian population, DR is more common and progresses more quickly than that seen in developed countries. I hypothesised that factors particular to this population alter the spectrum of disease to that observed in the West, and that these factors include anaemia, co-infection with HIV and poorly controlled blood sugar and blood pressure. I

suggested that the effects of these factors are mediated at the level of the endothelium; endothelial perturbation results in exhaustion of the mechanisms for endothelial self-repair leading to the development of maculopathy and ischaemic retinopathy.

4.7.4 Research questions addressed in this thesis

This thesis will address the following questions:

1. What is the prevalence, incidence and progression of grades of DR in patients attending diabetes clinics in Southern Malawi?
2. What are the determinants of severity and progression of DR in Southern Malawi?
3. Is there evidence of endothelial perturbation in patients attending diabetes clinics in Southern Malawi and is degree of endothelial dysfunction related to severity and progression of DR?

The Malawi Diabetic Retinopathy Study (MDRS) was designed to address the questions listed above. The design of this programme of research and the results from it are described in subsequent chapters.

Chapter 5. Malawi Diabetic Retinopathy Study: Aims, Design and Methodology

5.1 Aims of the chapter

In this chapter I describe the background, aims and methods of the Malawi Diabetic Retinopathy Study (MDRS), the study that I established to undertake the clinical work in my research fellowship and reported in this thesis.

5.2 Introduction

In Chapters 1 to 3 of this thesis I reviewed the pathophysiology of diabetic retinopathy (DR), grading schemes for DR and the existing evidence on the determinants of severity and progression of DR. I explained that in resource-poor Sub-Saharan Africa, population-specific variables, such as irregular drug supply, poor patient education and a high burden of infective disease and anaemia, are likely to affect the spectrum of pathology encountered. My systematic review of the epidemiology of DR in Africa (Chapter 4) highlighted the paucity of high quality epidemiological data about DR in Sub-Saharan Africa.

5.3 Background to the MDRS

To my knowledge, no cohort studies have investigated determinants of severity and progression of DR in Sub-Saharan Africa. The 2009 WHO Malawi national STEPwise survey estimated a prevalence of diabetes of 5.6% in adults 25-64 years, with similar prevalence in rural and urban areas [17]. In 2007 a pilot, cross sectional study was performed using clinical ocular examination to assess grades of DR in patients attending the diabetes clinic at Queen Elizabeth Central Hospital (QECH), Blantyre [2]. This study reported a high prevalence of sight threatening diabetic retinopathy (STDR) and proliferative retinopathy (PDR): 19.6% and 5.7%, respectively.

In response to these important findings I set out to elucidate the prevalence, incidence and progression of DR and visual impairment amongst adults attending diabetes clinics in Southern Malawi. This thesis describes the findings from the MDRS: a formal observational programme of research using a systematically sampled cohort, standardised clinical photography, independent grading at an accredited reading centre as well as data collected on covariates specific to this population. Data was collected on markers of endothelial function as part of a nested case control study.

5.4 Aims and objectives of the MDRS

The aim of the MDRS was to investigate the determinants of severity and progression of DR in Southern Malawi. The main hypothesis underlying this research is that, in the Malawian diabetic population, DR is more common, more aggressive and different in character than that seen in developed countries. It is likely that factors particular to this population alter the spectrum of disease to that observed in the West, and that these factors include anaemia, co-infection with HIV and poorly controlled blood pressure. I hypothesise that the effects of these factors are mediated at the level of the endothelium: endothelial perturbation and increased cell death results in exhaustion of the mechanisms for endothelial self-repair leading to the development of ischaemic retinopathy. This thesis outlines a program of both clinical and laboratory based research addressing the clinical determinants of DR severity and progression as well as the underlying cellular mechanisms.

The objectives of the MDRS were the following:

1. To investigate the prevalence, incidence and progression of DR in Southern Malawi
2. To investigate the risk factors for DR severity and progression in this population
3. To characterise endothelial function in Southern Malawian subjects with diabetes and to investigate relationships with severity of retinopathy

5.5 Design of the MDRS

The MDRS was a programme of research comprising 3 core components:

1. **Prospective cohort study of people with diabetes**

Section 5.9 (Methods) and Chapters 6, 7 and 8 (Results)

Prevalence, incidence and progression of DR were studied over a period of 24 months. Associations between grades of retinopathy and clinical and laboratory risk factors were investigated.

2. **Study of DR progression at 5 years**

Section 5.10 (Methods) and Chapter 9 (Results)

In 2007 a cross sectional study was performed of 281 patients attending the QECH diabetic clinic. This cohort was traced and re-examined at 5 years.

3. **Case-control study of endothelial function in Malawian subjects with diabetes**

Section 5.11 (Methods) and Chapter 10 (Results)

A subset of subjects from the cohort study (1) was investigated. Endothelial function was studied in 4 groups: subjects with diabetes and STDR, subjects with diabetes and DR but not STDR, subjects with diabetes but without DR and subjects without diabetes.

5.6 Setting

QECH in Blantyre is the only teaching hospital in Malawi. It provides primary and secondary care to the population of greater Blantyre (approximately 1.0 million, 50% adult), an urban and semi-urban population, and tertiary care to the Southern region of the country (approximately 6.0 million). In Southern Malawi primary care for diabetes is non-existent at health centre level. Therefore primary care for diabetes is delivered at district hospitals. Neither Blantyre nor Zomba have a district hospital so the central hospitals have to provide this service.

The diabetes clinic at QECH provides predominantly primary diabetes care to approximately 2,000 patients. The clinic represents the most specialised care in the public sector available to persons with diabetes in Malawi. Zomba Central Hospital

(ZCH) is approximately 60 miles from Blantyre. It provides primary and secondary care to the urban, semi-urban and rural population of Zomba district (approximately 500,000 people). In 2011 the newly established diabetes clinic served approximately 250 patients. Many of this population were newly diagnosed with diabetes and had received no previous eye screening or treatment. 2010 saw the opening of the Lions Eye Hospital attached to ZCH.

An important component of health care in Malawi is the 'health passport'. This booklet is carried by all patients in Malawi and contains details of medical interventions received from a variety of facilities and specialties. For most out-patient care this document is used in place of conventional hospital records. In the MDRS the health passport was used to acquire details of past medical history, to make referrals to other specialties including medicine, surgery and physiotherapy, to document brief details of diagnosis and treatments performed and to record dates of follow-up clinics.

5.7 Data management

The MDRS utilised a custom-made 'REDCap' database hosted at the Malawi Liverpool Wellcome Trust (MLW) Clinical Research Programme. REDCap (Research Electronic Data Capture, Vanderbilt University, Tennessee, USA) is a secure, web-based application designed to support data capture for research studies. The system provides audit trails for tracking data manipulation. Each study subject was allocated a unique identifier. Data on subject demographics, physical examination including visual acuity (VA) and point of care tests were manually recorded by the MDRS study team onto specially designed, paper-based data collection forms. I recorded clinical DR grading onto paper-based forms originally designed for the Liverpool Diabetic Eye Study (LDES) [94].

Data from the paper forms was entered onto the study database by Chrissy Pindani (study Research Nurse). Once the data was entered a series of steps were taken to check for transcription errors. Data entry for each data form was checked by me

with 100% quality assurance on critical variables. Once the dataset for a particular analysis was complete I performed manual (visual) and automated (STATA) checking of critical variables. Outliers thus identified (defined as a value that is more than 3 standard deviations from the mean) were then checked manually against original data forms. A number of minor errors were found and corrected before analysis.

Laboratory results were received in a web based format via the Prelink Laboratory Information Management System (Prelink LIMS, Johannesburg, SA). I entered these results into the study database. In a similar fashion I entered into the database results from ELISA assays and peripheral artery tonometry testing. The database was stored on centrally managed servers that were protected against data loss. Data was extracted from the database for analysis into either Microsoft Excel (Microsoft, Seattle, Washington USA) or STATA version 12 (StataCorp, Texas, USA).

5.8 Ethical approval

The MDRS received ethical approval from Liverpool School of Tropical Medicine Research Ethics Committee (protocol number: 11.88) and the College of Medicine Research Ethics Committee (COMREC) in Malawi (protocol number: P.08/11/1115). There were several subsequent amendments to address minor study modifications. See Appendices 3A-C and 4A-D for details of the consent forms and patient information sheets in English (Chichewa translations were also used but are not included in this thesis). Written informed consent was obtained from all subjects.

MDRS CORE COMPONENTS

5.9 Prospective cohort study of persons with diabetes

5.9.1 Subjects

Systematic random sampling was used to select subjects from the general diabetes clinics at QECH and ZCH (the only public sector diabetes clinics in Blantyre and Zomba) between December 2011 and May 2012. Patients attend these clinics for medical management of diabetes; no eye care is provided. The first subject was selected from the first 6 patients in the diabetes clinic line using marbles in a bag numbered 1 to 6. Then every 6th individual was approached until ten subjects were selected (the maximum number which could be assessed in a morning). Inclusion criterion was a diagnosis of diabetes according to American Diabetes Association (ADA) criteria [123] (Box 5.1).

Glycosylated haemoglobin (HbA1c) testing is not available in the public sector in Malawi. Therefore, in practice, inclusion criterion was diagnosis of diabetes according to ADA criteria numbers 2, 3 or 4. Exclusion criteria were age less than 18 years, first visit to the diabetes clinic, and diagnosis of gestational diabetes according to ADA criteria [123]. As described above, the diabetes clinics at QECH and ZCH provide predominantly primary diabetes care (primary care for diabetes does not exist at health centre level and there are no district hospitals in Blantyre and Zomba). Central hospitals are tertiary centres which receive referral cases. In order to effectively exclude referral cases from the MDRS cohort, patients living more than 60km from the clinic in question were excluded.

Box 5.1 American Diabetes Association criteria for diagnosis of diabetes [123]

1. HbA1C $\geq 6.5\%$. The test should be performed in a laboratory using a method that is National Glycohaemoglobin Standardisation Program (NGSP) certified and standardised to the Diabetes Control and Complications Trial (DCCT) assay*.
2. Fasting plasma glucose ≥ 126 mg/dl (7.0mmol/l). Fasting is defined as no caloric intake for at least 8 h*.
3. 2-hour plasma glucose ≥ 200 mg/dl (11.1mmol/l) on oral glucose tolerance testing. The test should be performed as described by the World Health Organisation, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water*.
4. In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose ≥ 200 mg/dl (11.1mmol/l).

*In the absence of unequivocal hyperglycaemia, criteria 1-3 should be confirmed by repeat testing.

5.9.2 Classification of type 1 and type 2 diabetes

Type 1 diabetes was diagnosed when subjects had commenced insulin therapy within 2 weeks of diagnosis and 2 of the following 4 features were present: age ≤ 19 years at diagnosis; BMI $\leq 25\text{kg/m}^2$; ketones 2+ on urinalysis; symptoms ≤ 4 weeks duration. Type 2 diabetes was diagnosed in subjects stabilised on oral medications or diet from diagnosis (fasting blood sugar (FBS) $\leq 130\text{mg/dL}$ on two occasions within 3 months). For subjects not fulfilling the above criteria, diagnosis of type 1 or type 2 diabetes was decided by two clinicians (PB and TA) with reference to clinical notes. Subjects with type 2 diabetes were sub-classified based on treatment: insulin requiring with or without oral hypoglycaemic agents, oral hypoglycaemic agents alone or dietary measures alone.

Criteria for the diagnosis of diabetes have been extensively debated [123,297]. Differentiating type 1 and type 2 diabetes can be difficult even with extensive laboratory investigations (including serum C-peptide and islet cell autoantibodies)

which were not available to this study [123,298]. There are issues with classification of diabetes which are specific to Africa. The mean age of onset of type 1 diabetes is reported to be higher than other regions [195]. Other phenotypes of diabetes are recognised including ‘ketosis prone atypical diabetes’ and ‘malnutrition-related diabetes’ [195,299] (Box 5.2). While our criteria are study specific they are similar to definitions used in large population based studies of DR [293,300-305] while taking into account the regional context.

Box 5.2 Summary features of ‘ketosis prone atypical’ and

‘malnutritional-related’ diabetes [after reference 195]

Ketosis prone atypical

Ketotic presentation

Children or young adults

3:1 male excess

Islet autoimmunity rare

Often strong family history

Remission possible

Malnutrition-related

Insidious onset

Young adults

2:1 Male excess

Occasional ‘type 1’ HLA pattern

Past or present malnutrition

Steatorrhoea in some areas

5.9.3 Clinical assessment

A standardised case record form was completed by the study team by questioning subjects and by reference to the ‘health passport’ carried routinely by patients in Malawi. Information was obtained about demographic details, date of diagnosis of diabetes, as well as anti-diabetic, anti-hypertensive, and anti-retroviral (ART) medications. Assessment of past medical history included presence or absence of stroke, ischaemic heart disease, neuropathy, foot ulcers, amputation, erectile dysfunction, previous diagnosis of tuberculosis, previous diagnosis of syphilis, diagnosis of malaria within the past year, and previous eye examination. Smoking was defined as current, former or never.

Physical examination was undertaken by a trained nurse or trained research assistants who were part of the study team. Blood pressure was measured using the United Kingdom Prospective Diabetes Study (UKPDS) protocol [14] (HEM-907 XL, Omron, Lake Forest, IL). Subjects were classified as hypertensive according to the WHO definition [17]: subject either taking antihypertensive medication, or systolic blood pressure (sBP) ≥ 140 mmHg, or diastolic blood pressure (dBp) ≥ 90 mmHg. Weight (Seca 875, Birmingham, UK) and height were recorded. VA (uncorrected and using pinhole) was measured as the number of letters read on a standard Early Treatment of Diabetic Retinopathy Study (ETDRS) chart (Sussex Vision, UK) using a standard protocol (testing at 4 metres initially and then at 1 metre if < 20 letters are read at 4 metres). For illiterate subjects a 4m logarithm of the minimum angle of resolution (logMAR) 'Tumbling E' chart was used (Sussex Vision, UK). For each subject with VA in the better eye < 80 letters I recorded what was, according to my clinical judgement, the primary cause of visual impairment. Causes of visual impairment were classified as DR, cataract, DR and cataract, age-related macular degeneration, glaucoma and 'other'.

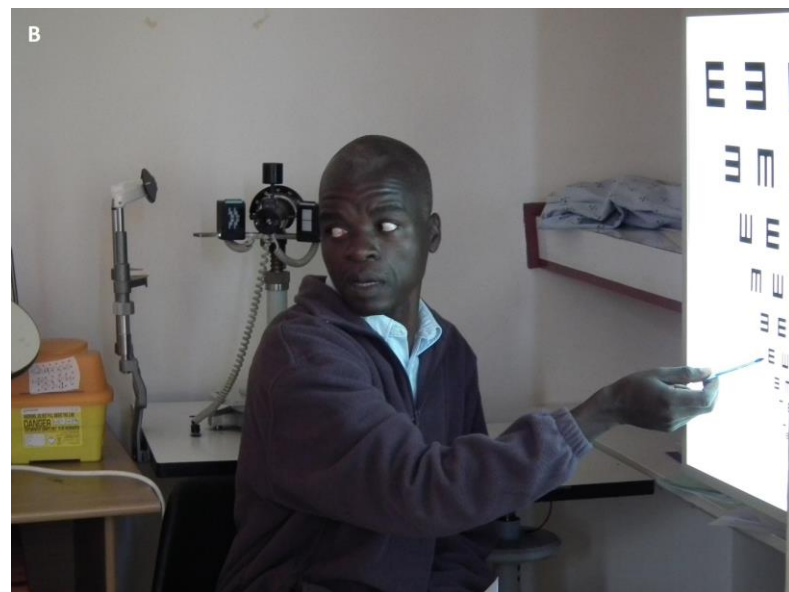
HIV status was defined as 'unknown', 'known HIV positive not taking ART', 'known HIV positive taking ART', or 'known HIV negative' (documented negative HIV test within 1 month). All subjects in the first 2 categories were offered HIV point of care testing according to Malawian national protocol [306] (Determine Rapid Test, Abbott, Hoofddorp, the Netherlands; Uni-Gold Recombigen, Trinity Biotech, Bray, Ireland; Bioline, SD, Korea). Those subjects diagnosed with HIV were referred to the dedicated HIV clinic at QECH from which ART is available. Haemoglobin was measured with a point of care test (Hb301, HemoCue, Angelholm, Sweden). Thresholds for anaemia were set according to WHO guidelines: 13.0g/dL for men; 12.0g/dL for women [307].

Blood samples were assayed for putative biochemical risk factors: fasting glucose, triglycerides, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, serum creatinine, and urine albumin-creatinine ratio (ACR)

(colorimetric assays performed at Malawi Liverpool Wellcome laboratories, Blantyre, Malawi using the Synchron CX5, Beckman Coulter, CA). Glycosylated haemoglobin (HbA1c) was measured using Boronate affinity chromatography performed at Norfolk and Norwich University Hospitals Laboratories, UK. A detailed description of HbA1c measurement in the MDRS is given below in Section 5.12. Hypercholesterolaemia was defined according to the WHO as ≥ 5.0 mmol/L [17]. Photographs of the MDRS study team assessing subjects are shown in Figure 5.1.

Figure 5.1 Photographs of the MDRS study team assessing study subjects.

Photograph A: Sister Chrissy Pindani taking a venous blood sample. Photograph B: Research Assistant Moffat Chidzuwa measuring visual acuity using a 4m logMAR 'Tumbling E' chart.



5.9.4 Assessment of retinopathy

Digital fundus photography of four 45° standard fields [94] with a stereo macular image was performed through dilated pupils (guttae tropicamide 1% and phenylephrine 2.5%) using CR6 fundus cameras (Canon, Reigate, UK). Photographic fields used in the MDRS are shown in Figure 5.2. MDRS standards for field position and quality are shown in Figure 5.3. Dual grading of photographic images was performed by accredited graders at the Liverpool Ophthalmic Reading Centre (an accepted reference standard for grading of retinopathy). In the event of disagreement between graders arbitration was performed by a senior ophthalmologist accredited in grading. Graders followed protocols established in Liverpool. Briefly, images were graded on graphic quality monitors against photographic standards defined for the ETDRS on standardised data forms in sessions lasting a maximum of 2 hours before a break, to ensure adequate quality.

In addition to fundus photographs, all subjects were examined by me (PB) using slit-lamp biomicroscopy. Cataract was graded according to the Lens Opacities Classification System (LOCS) III [308] and considered clinically significant when graded at ≥ 3 in any category (nuclear opalescence, nuclear colour, cortical or posterior subcapsular). A photograph of the MDRS study team performing retinal photography is shown in Figure 5.4.

Figure 5.2. Photographic fields used in the MDRS. Digital fundus photography of four 45° standard fields with a stereoscopic macula image were performed.

Illustrations show the right eye. Circle indicates the optic disc; cross indicates the centre of the fovea.

Field 1: Disc centred image. **Field 2:** Macular centred stereo image (2 images: a and b).

Field 3: Superior temporal image. Positioned with the disc in the 5 o'clock or 7 o'clock positions for upper temporal quadrant in the right and left eyes,

respectively. All of the disc should be visible with the disc margin abutting the edge of the image.

Field 4: Inferior temporal image. Disc in the 1 o'clock or 11 o'clock positions for the lower temporal quadrant in right and left eyes, respectively.

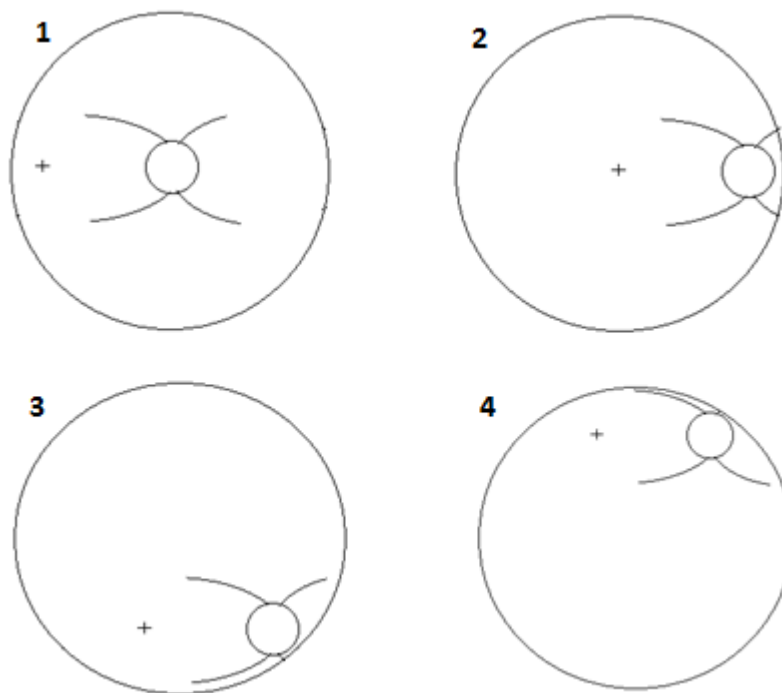




Figure 5.3. Standards for photograph field position and image quality used in the MDRS

A. Disc and macular centred images

Definitions for disc and macular centred images are taken from the English National Screening Programme [309]. A combined assessment of field position and image quality was made as follows.

Good

Macular image	Disc image
Centre of fovea $\leq 1DD$ from centre of image AND Vessels clearly visible within 1DD of centre of fovea AND Vessels visible across $>90\%$ of image	Centre of disc $\leq 1DD$ from centre of image AND Fine vessels clearly visible on surface of disc AND -Vessels visible across $>90\%$ of image
	

Adequate (Fair)

Macular image	Disc image
Centre of fovea $>2DD$ from edge of image AND Vessels visible within 1DD of centre of fovea	Complete optic disc $>2DD$ from edge of image AND Fine vessels visible on surface of disc

In some unusual cases (particularly in patients with a large disc), an image may fall within both good and adequate categories above. In such cases, the image was classified as good.

Inadequate (ungradeable; poor)

Failure to meet the definition of adequate above.

B. Upper temporal and lower temporal image quality

The following definitions are specific to the MDRS.

Good

Complete disc visible AND

Disc at 1.30 or 4.30 or 7.30 or 10.30 for right or left, inferotemporal or supereotemporal images, respectively AND

Fine vessels clearly visible on surface of disc AND

Vessels visible across >90% of image

Adequate

Some portion of optic disc visible AND

Disc within 1 DD of correct position AND

Fine vessels visible on surface of disc AND

Vessels visible across 50% of image

Inadequate

Failure to meet the definition of adequate above

Definitions of disc, fovea, 1DD for purposes of field position

Definitions taken from the English National Screening Program [309]. The image shown below is a perfectly aligned macular view of the right eye. The fovea lies at the centre of the image and is marked by a '+' symbol.



Figure 5.4 Photograph of Ophthalmic Clinical Officer Owen Mkangadzula performing retinal photography



5.9.5 Grading of retinopathy

Retinopathy and maculopathy were classified by feature specific grading using definitions established in the Liverpool Diabetic Eye Study (LDES) [94]. The LDES protocol was produced in 1991 and involves a simplification of the Wisconsin grading system. This in turn was based on the modified Airlie House classification used in the ETDRS [86] and is based on standard photographs. LDES definitions can therefore be mapped to ETDRS disease steps. Definitions of clinical features used in the ETDRS are shown in Appendix 1. In the LDES changes were made to definitions of retinopathy grades on discussion between the LDES team and the Wisconsin Reading Centre team (SP Harding personal communication) with reduced weighting of cotton wool spots, increased weighting of venous signs and intra-retinal microvascular anomalies (IRMA), exclusion of exudates outside the macula and no distinction made between small haemorrhages and microaneurysms [94] (Table 5.1).

Each of eight clinical features of retinopathy is graded by greatest degree in any field and an overall retinopathy score assigned. Grading of maculopathy is based on the presence of exudates and/or oedema in the macular region. Macular oedema is

assessed according to the ETDRS criteria for clinically significant macular oedema (CSMO) which is a stage of exudative maculopathy directly threatening or involving the fovea [86] (Box 5.3). In the LDES and in the MDRS, STDR was defined as any of the following: moderate pre-proliferative retinopathy or worse (level 40-71+); macular exudates in a circinate pattern or within one disc diameter of the foveal centre, or CSMO (level 3-4: sight threatening maculopathy); or other diabetes related retinal vascular disease: central or branch retinal artery occlusion, central or branch retinal vein occlusion.

For the purposes of analysis, for each feature of DR photographic grading took precedence over clinical examination. If any feature was classified as 'ungradeable' on photographic grading the biomicroscopy grade for that feature was used. Only if a feature was deemed 'ungradeable' on both photographic and clinical grading was 'cannot grade (CG)' recorded as the final grade for this feature. If a single feature was graded CG (most likely with IRMA) the following algorithm applied:

1. If a feature higher on the LDES scale was present then this took precedence and a grade was assigned
2. If no higher feature was present then the retinopathy grade for that eye was assigned based on the features which could be graded ('minimum grade')

Box 5.3. Definition of clinically significant macular oedema (CSMO) as used in the Early Treatment of Diabetic Retinopathy Study (ETDRS) [86]

1. Retinal thickening within 500µm of the foveal centre
2. Exudates within 500µm of the foveal centre which are associated with adjacent retinal thickening
3. Retinal thickening at least 1 disc area in size any part of which is located within 1 disc area of the foveal centre

Table 5.1 Levels of retinopathy and maculopathy in the Liverpool Diabetic Eye Study [94]

Level	Definition
Retinopathy	
11	No retinopathy
12	Questionable
20	Haemorrhages or microaneurysms < ETDRS standard photograph 2A †
30	Haemorrhages or microaneurysms ≥ ETDRS standard photograph 2A, and/or 1-6 cotton wool spots
40	Haemorrhages/ microaneurysms ≥ ETDRS STD 2A and/or ≥ 6 CWS; and/or 1 quadrant venous changes; and/or IRMA < ETDRS STD 8A
50	IRMA ≥ ETDRS STD 8A and/or 2 or more quadrants venous changes and/or pre-retinal haemorrhage in absence of proliferation
60	Fibrovascular proliferation and/or proliferative retinopathy
70	Diabetic Retinopathy Study high risk characteristics
71	Tractional retinal detachment
72	No fundal view due to vitreous blood
90	Ungradeable due to any other reason e.g. media opacity
Maculopathy	
0	No maculopathy
1	Questionable: < 50% certainty of presence of exudate
2	Exudate >1 disc diameter (DD) from fixation
3	Circinate ring of exudates within macula >1 disc area in size but not within 1 DD of fixation
4	Exudates within 1 disc diameter of fixation and/or presence of clinically significant macular oedema
8	Exudates due to other diseases e.g. vein occlusion, choroidal neovascularisation
90	Ungradeable

† Definition of any diabetic retinopathy: ≥ 1 haemorrhages of microaneusyms (HMa) in either eye.

Flame shaped haemorrhages associated with hypertension are discounted.

5.9.6 Study schedule

Subject recruitment (Visit 1) took place at the diabetes clinics at QECH and ZCH between December 2011 and May 2012. 12 month follow-up (Visit 2) occurred between December 2012 and May 2013. 24 month follow-up (Visit 3) took place between December 2013 and May 2014. Table 5.4 gives a summary of data collected at each study visit. Subjects were recalled for follow-up visits by telephone. Subjects without telephones or whose telephone number changed between study visits were traced by the MDRS study team.

Tracing was systematic. The team attended the diabetes clinic at QECH and ZCH weekly between December 2012 and May 2013 and again between December 2013 and May 2014 to approach patients in the clinic waiting room. Subjects not found at the diabetes clinic were visited at home to offer an appointment (Figure 5.5). If the subject had relocated attempts were made to contact them via their church or place of worship. Finally the president and vice president of the patients' organisation, the Diabetes Association of Malawi were contacted in order to personally identify subject whereabouts.

Figure 5.5 Research Nurse Chrissy Pindani visiting a study subject at home to offer an appointment.



Table 5.4 Study schedule for the MDRS cohort study: data collected at each patient visit

Data collection method	Data collected / test performed	Visit number		
		1	2	3
Case record form	Demographic details	X	X	X
	Past medical history	X	X	X
Physical examination	BP, height and weight	X	X	X
	Visual acuity	X	X	X
Point of care tests	Hb; HIV VCT	X	X	X
Laboratory biochemical tests	HbA1c; urine ACR	X	X	X
	TG, LDL and HDL	X		
Ophthalmology	Retinal photograph	X	X	X
	Clinical grading of DR	X	X	X

BP = blood pressure; Hb = haemoglobin; HIV VCT = human immunodeficiency virus voluntary testing and counselling; LDL = low density lipoprotein cholesterol; HDL = high density lipoprotein cholesterol; TG = triglycerides; HbA1c = glycosylated haemoglobin; ACR = albumin creatinine ratio.

5.9.7 Study outcomes

The primary outcome of the cohort study was progression of DR by 2 or more steps on the LDES severity scale. '2 step progression' equates to either 1 step progression in one eye and 1 step progression in the other eye *or* 2 step progression in one eye.

Secondary outcome variables at baseline were:

1. Prevalence of all grades of retinopathy
2. Prevalence of STDR
3. Associations of systemic variables with STDR
4. Prevalence of moderate and severe visual loss as defined by WHO [310]

Secondary outcome variables at 12 and 24 month follow-up were:

1. Incidence of retinopathy
2. Progression of DR by ≥ 3 steps on the LDES Severity Scale
3. Progression to STDR

4. Progression to sight threatening maculopathy
5. Development of DR necessitating scatter laser
6. Development of maculopathy necessitating macular laser
7. Composite end point of either progression of DR by 2 or more steps on the LDES scale or development of DR necessitating scatter laser
8. Associations of systemic variables with progression of retinopathy by ≥ 2 steps, development of DR necessitating scatter laser, and the composite end point of either progression of DR by 2 or more steps on the LDES scale or development of DR necessitating scatter laser
9. Loss of 5 letters on the ETDRS visual acuity scale
10. Loss of 15 letters on the ETDRS visual acuity scale
11. Progression to moderate and severe visual loss as defined by WHO [310]

Development of DR necessitating scatter laser is defined as 'listed for scatter laser after the first study visit and up to and including the final study visit' (i.e. this endpoint cannot be achieved twice). Development of DR requiring laser treatment does not include those listed for scatter at baseline. Similarly development of maculopathy necessitating macular laser is defined as 'listed for macular laser after first study visit and up to and including the final visit.

5.9.8 Sample size calculation: longitudinal data

This study was powered for the primary endpoint (2 years): progression of retinopathy by 2 or more steps on the LDES scale. On the basis of previous studies [83,84,190] I anticipated 20% progression over the 2 year study period. I intended to use multiple logistic regression analysis (mixed effects) to analyse the effect on progression of retinopathy of six variables: sBP, HbA1c, Hb, HIV, urine ACR, and LDL cholesterol. In logistic regression analysis, as a rule of thumb, the number of the least common of the two possible outcomes (in this case progression of DR by 2 or more steps) divided by the number of predictor variables should be at least 10.

Three hundred subjects would give 60 (20%) with progression and therefore with 6 variables the rule described above holds. Allowing for loss to follow-up and mortality of 20% gives **360 sample size**. My sample size calculation was based on logistic regression analysis of data from two time points: start and end of the trial. The MDRS cohort underwent 3 assessments: baseline, 1 year and 2 years allowing greater power of analysis. The correlation between data from the same subject at several time points was taken into account using mixed-effects terms in the logistic model.

5.9.9 Sample size calculation: cross sectional data

I performed the following sample size calculation to ensure that the sample of 300 subjects estimated above, based on the logistic regression analysis, would also be sufficient to identify a significant difference in the proportion of subjects with STDR at baseline according to a single clinical variable. The example of HIV is used here as, based on our group's recent cross sectional study [2], the percentage of HIV positive patients is expected to be 15% (i.e. small numbers relative to other variables).

I used the chi-squared (χ^2) test with unequal sample sizes for the study to be powered with 90% power to detect 25% difference in prevalence of STDR between subjects with and without HIV at the 5% level of significance. The expected prevalence of STDR in a cohort based on our recent cross sectional study [2] was 20%. Therefore, a prevalence of STDR of 45% for the population of patients with HIV was considered to show a clinically significant change. Since the expected prevalence of HIV in the cohort was 15%, the ratio in sample sizes between the two groups was estimated to be 3/17, and the sample size needed (nQuery Advisor) was $n_1=43$ subjects with HIV and $n_2=238$ subjects without HIV, giving a total sample size of **280 subjects**.

5.9.10 Statistical analysis

I performed all of the statistical analysis personally. Support from one of my supervisors Dr Marta Garcia-Finana from the Department of Biostatistics, University of Liverpool was used to verify the data analysis. An *a priori* analysis plan was followed. Grades of DR were calculated by patient according to the worse or only gradeable eye. Retinopathy and maculopathy were graded separately by worse eye i.e. the worse grade for retinopathy and the worse grade for maculopathy could be taken from different eyes. Visual acuity data were investigated by patient according to the better eye.

For normally distributed continuous data, mean and standard deviation (SD) were calculated. For continuous data which was not normally distributed median and interquartile ranges were calculated. Frequencies were quoted for categorical data. 95% confidence intervals (CIs) were calculated for proportions. When comparing 2 groups (numerical data) with independent measurements the unpaired t-test was used when data was normally distributed and the Wilcoxon rank sum test when data was not normally distributed. For categorical data Fisher's exact test was used when comparing 2 categories and 2 groups of independent measurements. The chi-squared (χ^2) test for trend was used to analyse trends across >2 groups. All tests were two-sided and data were considered significant when $p < 0.05$.

When assessing cumulative and annual incidence at 24 month follow-up (visit 3), study subjects with baseline retinopathy data and at least one subsequent visit were analysed. Cumulative and annual incidence rates of grades of DR and STDR were calculated for one year intervals using the life table method. The life-table method allows for censored data taking into account varying intervals of follow-up after the first study visit. Subjects who had not developed DR, STDR or other outcome threshold contribute to person-years of follow up until their last study visit. Separate life table calculations were performed for each baseline grades of retinopathy progressing to each different endpoint (e.g. any retinopathy, level 20 DR, level 30 DR, STDR, etc.). The number of patients at each time interval and the

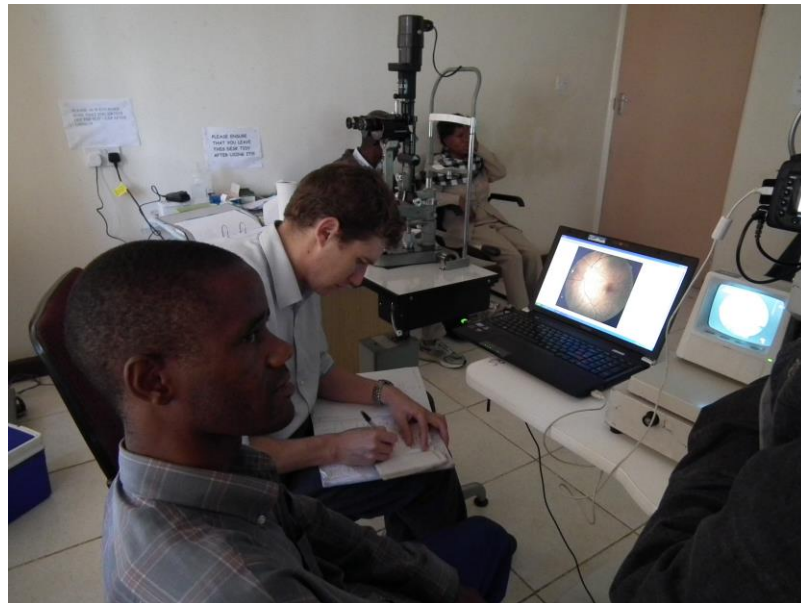
number reaching endpoint at each time interval are included in the life tables. All calculations were performed using STATA version 12 (StataCorp, Texas, USA). Details of specific analyses are given in the relevant results chapters.

5.9.11 Treatment of retinopathy

Subjects with STDR who met thresholds for either scatter (peripheral retinal photocoagulation) or macular argon laser treatment, were treated by me in a linked dedicated laser clinic. Threshold for scatter laser treatment was the ETDRS '4-2-1' rule (4 quadrants of haemorrhages and microaneurysms (HMa) \geq ETDRS standard 2A, *and/or* 2 quadrants of venous beading \geq standard 6A, *and/or* 1 quadrant of IRMA \geq standard 8A). Standard treatment was approximately 3000 burns placed approximately one burn width apart outside the arcades and 2 disc diameters from the centre of the fovea, keeping 500 μ m from the nasal margin of the disc. Scatter treatment was applied in 2 sessions if no maculopathy was present or 3 sessions if maculopathy was present (fractionated treatment).

Threshold for macular laser was CSMO as defined in Box 5.3 and visual acuity less than 80 ETDRS letters (equivalent to 6/9 Snellen). Macular laser was also performed for 'sight threatening exudates' defined as thick or streak exudates close to the foveal centre but not meeting the definition of CSMO. Macular laser was performed according to the modified grid technique [311]: 'direct treatment' of microaneurysms in areas of retinal thickening (avoiding microaneurysms within 500 microns from the centre of the fovea), and 'grid treatment' (barely visible burns 2 burn widths apart) applied to areas of thickening and not treated during the direct microaneurysm treatment. Treatment and response to treatment was recorded as part of the MDRS. Subjects with other treatable ophthalmic or systemic pathology were either treated by me or referred to the appropriate service at QECH or ZCH. Figure 5.6 shows a photograph of a patient being counselled for laser treatment.

Figure 5.6 Photograph of the author and Research Assistant Moffat Chidzuwa (out of shot right) counselling a patient with reference to their retinal photographs



5.10 Study of DR progression at 5 years

5.10.1 Background to the study of DR progression at 5 years

A cross sectional study of complications of diabetes was conducted at the QECH diabetes clinic in 2007 [1]. Of 620 subjects included in the study 281 were examined for retinopathy by an ophthalmologist. The results of this sub-study have been published [2] and are referred to in Chapter 4 of this thesis. In 2007 laser treatment was not available in the public sector in Blantyre. A frequency doubled neodymium-doped yttrium aluminium garnet (Nd:YAG) laser (Purepoint Alcon Texas) was donated to Lions First Sight Eye Unit in Blantyre in 2010, by the World Diabetes Foundation. However, few patients were treated in 2010 or 2011. Recall of the 2007 subjects would be a valuable opportunity to assess progression of retinopathy and visual loss over 5 years in a population not exposed to laser treatment.

In this part of the MDRS I aimed to trace as many of the 2007 subjects as possible, document grades of DR and thereby assess progression of retinopathy over 5 years, report associations of progression and document visual loss and causes of visual loss in an untreated cohort. The results of this study are presented in Chapter 9 of this thesis.

5.10.2 Setting: changes at the QECH diabetes clinic between 2007 and 2012

In the period 2007 to 2012 the clinic underwent a number of changes. The number of registered patients increased from approximately 800 to 2000. A vibrant nurse-led patient education programme supported by the World Diabetes Foundation commenced in 2008. Its aims are to improve compliance with diet and medications and educating patients on the complications of diabetes. An electronic records system (Diabetes and Hypertension System, Baobab Health Trust, Malawi) was

installed in early 2010. This runs in parallel to the 'health passport' system described above.

In 2007, medications regularly available free of charge were glibenclamide and insulin (lente and soluble). Metformin was available from private pharmacies but rarely from the hospital pharmacy. By 2011 metformin was more frequently available free of charge. However, supplies of all drugs remained intermittent. Tests available at the clinic were the same in 2007 as 2012. Glycaemic control was measured by fasting blood sugar (FBS) on the day of clinic and blood pressure, height and weight was measured by nursing staff. Measurement of lipids, glycosylated haemoglobin and urine test sticks for microalbuminuria were not available routinely.

5.10.3 Subjects

Consecutive subjects attending for routine out-patient review between March and June 2007 were invited to participate in the original cross sectional study of complications of diabetes, reported elsewhere [1]. Of 620 subjects included in the study 281 were examined for retinopathy by an Ophthalmologist [2]. Sampling was *ad hoc*: subjects had slit lamp examination if the Ophthalmologist was present at the particular clinic at which they were recruited.

5.10.4 Tracing of subjects from the 2007 cohort

The 2007 study was not planned as a cohort study. Patient names and date of birth were recorded and a sticker placed in their health passport. No contact details were recorded. The population of Blantyre is extremely fluid. Migration levels between rural and urban environments and between towns are known to be high. It is common for people who become disabled (for example as a result of a stroke) to relocate to be cared for by family members. Under normal circumstances no attempt is made to contact subjects who fail to attend a clinic appointment. Although no formal study has assessed mortality rate in the QECH diabetes clinic population, it is believed to be high. Many persons die at home and there is no

system for registering deaths. Subject tracing was therefore expected to be extremely difficult.

A systematic approach to tracing of subjects was developed. All subjects recruited to the MDRS main cohort were asked about participation in the 2007 study and their health passports checked for the study sticker. The QECH diabetes clinic electronic patient record system was searched by patient name by the MDRS study team. Identified subjects were then contacted by phone. The MDRS study team attended the diabetes clinic weekly between May and November 2012 to approach patients in the clinic waiting room. Finally the President and Vice-president of the patient's organisation the Diabetes Association of Malawi reviewed the list of subjects from 2007 in order to personally identify subject whereabouts. Written informed consent was obtained from all subjects prior to enrolment.

5.10.5 Confirmation of subject death

Confirmation of subject death was attempted in a systematic manner. The relatives of subjects reported to be deceased were visited at home by the study Research Nurse between May and November 2012. The nurse was trained to record, on a standard form, brief written narratives from families or other reliable informants. If available the death certificate and the health passport were reviewed and cause of death and/or brief details of last illness recorded. A subject was recorded as dead if confirmed by a relative or 'Traditional Authority' (village leader in rural districts), or if a death certificate or marked grave was seen by the study nurse. I assigned a probable cause of death after reading the form.

5.10.6 Clinical assessment

In the 2007 study a self-reported questionnaire was completed with the assistance of a research assistant and with reference to the subject's 'health passport'. Data was collected on demographic details, diet, past medical history and medications. Diabetes with young age at onset and early use of insulin was deemed to be type 1 with all others as type 2. Subjects with type 2 diabetes were sub-classified based on

treatment: insulin-requiring with or without oral hypoglycaemic agents, oral hypoglycaemic agents alone or dietary measures alone. For the purposes of the analysis below the 2007 allocation of diabetes type was retained i.e. subjects were not reclassified in 2012.

In 2007 a physical examination by a trained clinician included blood pressure, height, weight and neurovascular assessment (abridged four point monofilament examination with a 10g monofilament [312]). Visual acuity (corrected with pin-hole) was measured using a Snellen chart (not, as in 2012, using an ETDRS chart). FBS, HbA1c and HIV status were tested. A subgroup of subjects were also tested for microalbuminuria and serum creatinine. In 2012 all subjects were assessed in the same manner as subjects of the main MDRS cohort (described above in Sections 5.9.3 to 5.9.6).

5.10.7 Assessment of retinopathy

In both 2007 and 2012, retinopathy and maculopathy were classified by feature specific grading using definitions established in the LDES [94]. In 2007 slit lamp biomicroscopic retinopathy grading with 90 and 60 dioptre lenses was performed by one experienced Ophthalmologist (Mr Simon Glover) [2]. Pupils were dilated with 1% tropicamide +/- 10% phenylephrine. There was no external validation procedure. In 2012 all subjects were assessed in the same manner as the main MDRS cohort (described above in Sections 5.9.4 and 5.9.5). The 2012 grading procedure was more robust: dual grading with arbitration of digital fundus photography of four 45° standard fields [94] performed by accredited graders at a recognised reading centre. Subjects with STDR who met thresholds for laser treatment were treated by me as described in Section 5.9.11.

5.10.8 Statistical analysis

The 2007 study was not planned as a cohort study. Therefore no power calculation was performed. General statistical methods are described above in Section 5.9.10. Details of specific analyses are given in results chapters.

5.11 Case-control study of endothelial function in Malawian subjects with diabetes

5.11.1 Background to the study of endothelial function

Endothelial dysfunction is implicated in the pathophysiology of DR. Adherence of leukocytes is associated with direct injury to and apoptosis of endothelial cells and with disruption of endothelial tight junctions [45]. This nested case-control study aimed to characterise endothelial function in Malawian subjects with diabetes and investigate relationships with severity of retinopathy.

5.11.2 Subjects

A subset of subjects from the main cohort study (Section 5.9) were investigated plus control subjects (without diabetes). I planned to study endothelial function in 4 groups each consisting of 40 subjects:

1. subjects with diabetes and STDR at baseline
2. subjects with diabetes and DR but without STDR
3. subjects with diabetes but without DR
4. control subjects without diabetes

All subjects recruited to the main cohort study were offered the chance to participate in the case control study until each of the above groups reached their recruitment goal (40 subjects in each).

5.11.3 Control subjects

Systematic random sampling was used to recruit control subjects (without diabetes) from spouses of patients attending the QECH diabetes clinic between May and June 2012. A list of registered patients was obtained from the clinic. The first subject was selected from the first 6 patients on the list using marbles in a bag numbered 1 to 6. This patient was telephoned and asked if their spouse wished to participate in the study. Then every 6th individual was approached until the quota was fulfilled. Inclusion criterion was being a spouse of a patient attending the QECH diabetes

clinic. Exclusion criteria were age < 18 years and a diagnosis of diabetes according to ADA criteria [123] (Box 5.1). Therefore control subjects with fasting blood glucose $\geq 7.0\text{mmol/l}$ or HbA1c $\geq 6.5\%$ were excluded from the study.

Control subjects underwent all the assessments of the main cohort study: demographic and medical history case record form (Section 5.9.3), physical examination including visual acuity (Section 5.9.3), point of care and biochemical tests (Section 5.9.3), and retinal photography and grading (Section 5.9.4 and 5.9.5). Additionally a venous blood sample was taken in order to test plasma levels of endothelial biomarkers and a peripheral artery tonometry measurement was made.

5.11.4 Serum markers of endothelial dysfunction

I measured plasma levels of 4 parameters of endothelial function: vascular endothelial growth factor (VEGF), soluble vascular cell adhesion molecule (sVCAM-1), E-selectin and soluble inter-cellular adhesion molecule-1 (sICAM-1). In addition levels of C-reactive protein (CRP) were measured by MLW laboratories. These parameters reflect inflammation (CRP, sICAM-1), endothelial dysfunction (E-selectin and sVCAM-1) and angiogenesis (VEGF), respectively.

Levels of VEGF, sVCAM-1, sICAM-1 and E-selectin were quantified using enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, USA). These assays employ a quantitative sandwich enzyme technique. Plasma samples were prepared by MLW technicians: whole blood samples were collected in ethylenediamine-tetra-acetic acid (EDTA) tubes and centrifuged for 15 minutes at 1000g within 2 hours of collection. Plasma was then aliquoted and stored at -20 degrees until ELISAs were performed. A standard of each of the molecules being measured (e.g. VEGF) was provided by the manufacturer of the ELISA kits. This standard was reconstituted with 1.0mL of calibrator diluent solution provided by the manufacturer. This stock solution was used to make a dilution series of the molecule in question. The dilution series served as a positive control and to

produce a standard curve from which to derive the concentration of each molecule in the plasma samples. Calibrator diluent solution served as a negative control.

Each standard, negative control and sample was processed in duplicate. VEGF ELISA is described below. ELISAs for other molecules differed only in the specific solutions used; all were supplied by the manufacturer of the test kits. 100µl of assay diluent was added to each well of a microplate provided in the ELISA kit. 100µl of standard, negative control or plasma sample was added per well. The microplate was then covered with an adhesive strip supplied in the kit and incubated for 2 hours at room temperature. Each well was then aspirated and washed 3 times with 400µl of manufacturer supplied wash buffer using an autowasher (ELx50 microplate strip washer, Biotek, Vermont, US).

200µl of VEGF conjugate was added to each well. Samples were covered and incubated at room temperature for 2 hours. Aspiration/wash was repeated as described above. 200µl of substrate solution was added to each well and incubated at room temperature for 25 minutes protected from light. 50µl of 2M sulphuric acid was added to each well. The optical density of each well was determined with 30 minutes using an absorbance microplate reader set to 450nm (ELx808, BioTek, Vermont, US). The concentration of each biomarker was calculated from the optical density using a standard curve. Readings from duplicate wells were averaged and the zero standard optical density (control) subtracted. A standard curve was created by plotting mean absorbance for each standard against concentration. The line of best fit was determined by regression analysis. The concentration of VEGF in each sample was determined using the regression formula.

5.11.5 Pulse amplitude tonometry

Digital pulse amplitude tonometry (PAT) was measured using the 'EndoPAT-2000' (Itamar, Israel). This device is a non-invasive method of evaluating endothelial function. A fingertip probe measures pulse wave amplitude at baseline, during brachial artery occlusion (by a blood pressure cuff inflated to suprasystolic

pressures for 5 minutes) and during reactive hyperaemia following cuff release. A second probe is placed on the contralateral finger to serve as a control. Prior to testing subjects were allowed to rest whilst seated for 15 minutes. Clothing which might restrict blood flow to the arms as well as jewellery and watches were removed. As per the manufacturer's instructions the room temperature was maintained at 21-24°C using an air conditioning unit. A blood pressure reading was taken from the control arm prior to testing. Subjects were positioned in a reclining position (45 degrees) for testing with the arms supported.

Probes were placed on the subject's index fingers. If the index finger was unsuitable the middle finger was used. Digital pulse wave amplitude was recorded for a resting baseline period of five minutes. A blood pressure cuff applied to the study arm was then inflated to 200 mmHg, or 50 mmHg above systolic blood pressure, whichever was greater. After five minutes arterial occlusion the cuff was rapidly deflated. Pulse wave amplitude was then recorded for five minutes. Post occlusion-pre occlusion ratio (reactive hyperaemia-PAT (rhPAT) index), Framingham reactive hyperaemia (FRHI), and augmentation index (a measure of arterial stiffness) were calculated automatically by an inbuilt computer algorithm. The EndoPAT software normalises the rhPAT index to the control arm to correct for changes in systemic vascular tone.

5.11.6 Statistical analysis

A basic *a priori* analysis plan was followed. However, the analysis was, by its nature, exploratory. In cross sectional analysis endothelial function was compared across 4 groups (described above in Section 5.11.2). Multiple linear regression models were used to compare subjects with and without diabetes, subjects with and without diabetic retinopathy and subjects with and without STDR with reference to five serum markers: CRP, ICAM-1, E-selectin, VCAM-1 and VEGF, in addition to rhPAT index, FRHI and augmentation index. I constructed a logistic regression model (backwards stepwise with probability of removal of 0.2) to determine the odds ratio

(OR) and 95% CIs for the presence of diabetes in association with an initial 8 variables: age, sex, CRP, VEGF, ICAM-1, VCAM-1, E-selectin, and rhPAT index.

In longitudinal analysis, endothelial function was compared between subjects with diabetes whose retinopathy progressed by 2 steps on the LDES scale and subjects whose retinopathy did not progress at 24 months. Baseline endothelial function was also compared between subjects who had died and those who survived at 24 months. Multiple logistic regression analysis (mixed effects) was used to analyse the effect on progression of retinopathy (and in a separate analysis on death) of an initial 8 variables: age, sex, CRP, VEGF, ICAM-1, VCAM-1, E-selectin, and rhPAT index. Further details of analyses performed in the endothelial sub-study are given in Chapter 10, Section 10.3.2.

5.11.7 Sample size calculations

The case control of endothelial function was powered for 2 endpoints at baseline: serum level of VEGF and rhPAT index. Using the unpaired t-test **40 subjects** in each group would be required to detect a difference of 100pg/ml in VEGF level. On the basis of previous studies I estimated that the standard deviation for VEGF level for subjects with diabetes but no retinopathy would be 100pg/ml [48]. A similar SD was assumed across groups. The power considered was 80% and the significance level 0.0167 (which is 0.05/3 to adjust for 3 comparisons using Bonferroni correction). Using the unpaired t-test **40 subjects** per group would also be required to detect a difference of 0.3 units in rhPAT index (assuming SD of 0.4) with power 80% and significance level 0.0167 (3 comparisons).

5.12 Measurement of glycosylated haemoglobin (HbA1c) in the MDRS

5.12.1 Issues encountered in HbA1c measurement

A number of issues were encountered in the measurement of HbA1c. A description of these difficulties, subsequent analysis and the action taken is given below. The original plan was to test all HbA1c samples at the MLW laboratories. MLW uses a turbidimetric immunoinhibition (colorimetric) assay. Delays to commencement of HbA1c testing at MLW meant that baseline samples from the main cohort were stored for approximately 1 year at -80 degrees prior to testing. A large number of very low and very high results were noted when testing commenced in November 2012. Therefore whole blood samples were sent to Norwich and Norfolk University Hospital, UK for boronate affinity chromatography testing: a method reported to be affected to a lesser degree by prolonged storage [313].

Samples were sent to Norwich from the following: all subjects in the MDRS main cohort at visit one (baseline visit); all subjects from the study of DR progression at 5 years; all subjects without diabetes (control subjects) from the case control study of endothelial function; and a 20% sample of subjects in the MDRS main cohort at visit 2 (12 months). Comparisons between samples tested at MLW laboratories and those tested at Norwich for subjects in the MDRS main cohort at visit one are shown in Table 5.5 and Figures 5.7, 5.8, 5.9 and 5.10. Table 5.6 shows a comparison between HbA1c measurements from MLW and Norwich laboratories from each of the 4 groups of subjects listed above.

Table 5.5 Comparison between HbA1c results from MLW and Norwich laboratories for subjects in the MDRS main cohort at visit 1 (baseline visit). STDR = sight threatening diabetic retinopathy.

Parameter	MLW	Norwich
Number of observations	302	351
Mean HbA1c	9.262252	7.81994
SD	4.352496	2.51909
Univariate logistic regression STDR	OR 1.04; 95% CI 0.98 – 1.10; p=0.159	OR 1.14; 95% CI 1.04 – 1.25; p=0.004

Figure 5.7 Scatter plot of fasting blood sugar (FBS) versus glycosylated haemoglobin (HbA1c) measured at MLW laboratories for subjects in the MDRS main cohort at visit 1 (baseline visit). Correlation: 0.3075.

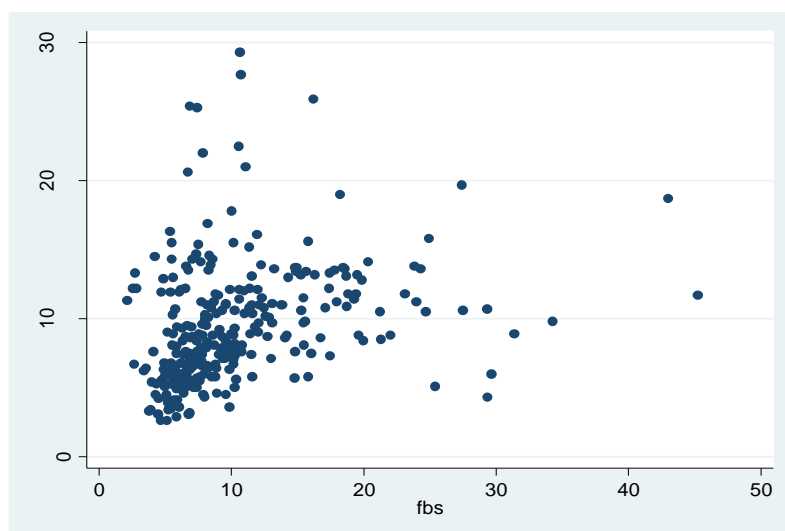


Figure 5.8 Scatter plot of fasting blood sugar (FBS) versus glycosylated haemoglobin (HbA1c) measured at Norwich and Norfolk University Hospital laboratories for subjects in the MDRS main cohort at visit 1 (baseline visit). Correlation: 0.6398.

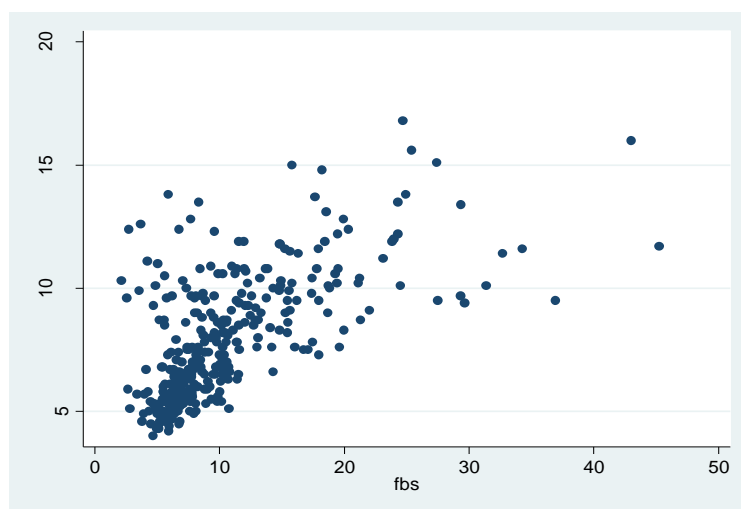


Figure 5.9 Scatter plot of HbA1c results from MLW and Norwich and Norfolk University Hospital laboratories for subjects in the MDRS main cohort at visit 1 (baseline visit). Correlation: 0.521.

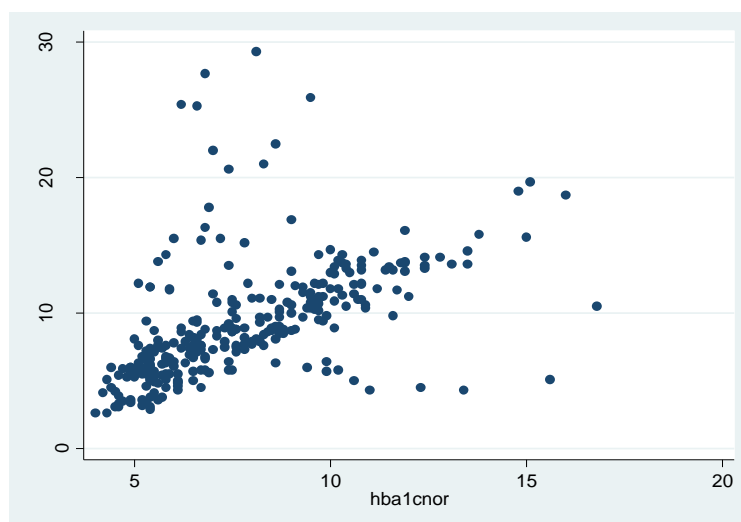


Figure 5.10 Bland Altman plot (differences between pairs of measurements plotted against mean of each pair) of HbA1c results from MLW and Norwich laboratories for subjects in the MDRS main cohort at visit 1 (baseline visit). Mean difference (MLW greater mean): 1.46 (95% CI 1.03-1.88). Range: 3.30-18.70. Pitman's Test of difference in variance: $r = 0.568$, $n = 301$, $p = 0.0001$

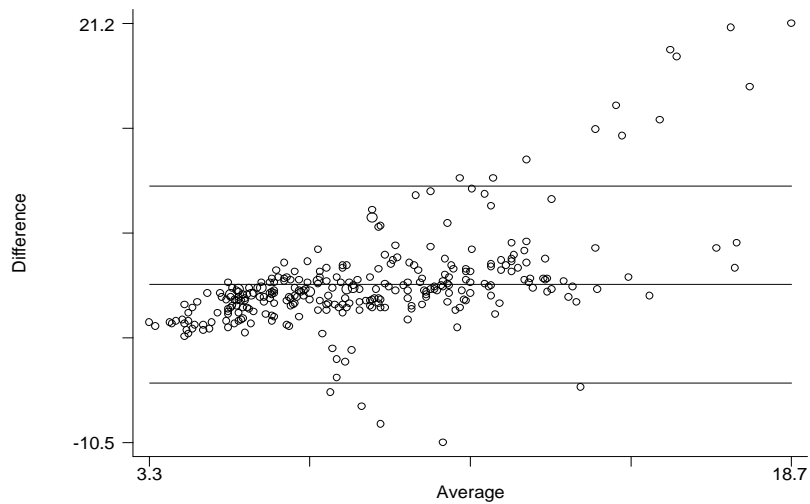


Table 5.6 Comparison of HbA1c measurements from MLW and Norwich laboratories categorised by subject group. STDR=sight threatening diabetic retinopathy. FBS=fasting blood sugar.

Parameter	Subject group							
	Main cohort visit 1		Five year cohort		Control subjects		Main cohort visit 2	
	MLW	Norwich	MLW	Norwich	MLW	Norwich	MLW	Norwich
Dates samples taken	Dec 2011-May 2012		May-Sept 2012		May-July 2012		Dec 2012 – Apr 2013	
Pre-test duration of storage (mths)	12	14	6	9	9	9	3	4
Number of observations	302	351	103	131	33	42	280	71
Mean	9.26	7.81	10.01	8.29	5.53	4.92	9.15	8.11
SD	4.35	2.52	4.08	2.32	1.20	0.68	2.94	2.62
Univariate regression STDR OR (95% CI)	1.04 (0.98 – 1.10)	1.14 (1.04– 1.25)	1.06 (0.97- 1.17)	1.16 (0.99– 1.35)	NA	NA	1.15 (1.06- 1.26)	1.18 (0.97 – 1.43)
Correlation FBS	0.308	0.640	0.40	0.64	0.603	0.812	0.499	0.732
Correlation MLW vs Norwich	0.52		0.64		0.59		0.88	
Bland-Altman mean difference	1.46		1.76		0.59		1.62	

5.12.2 Summary of comparisons between laboratories and action taken

HbA1c measurements from MLW laboratories showed higher means and greater standard deviation (SD) than those from Norwich. Estimates of SD from samples tested at Norwich remained relatively constant between the different groups of subjects while estimates of SD from MLW were greater for earlier samples. Differences in means and SD within subjects groups were smaller in later samples which may be explained by reduced storage times prior to testing. HbA1c measurements from MLW showed a weaker correlation with fasting blood sugar and a less strong association with STDR. The strength of association (correlation) between MLW and Norwich was reasonable and better for later (main cohort visit 2) samples. From the Bland Altman plots it is clear that MLW measurements tended to be higher than those from Norwich. There was a tendency for more large positive differences than large negative ones (positive skew) and a tendency for greater positive differences with greater HbA1c.

In conclusion, HbA1c measurements from Norwich appeared to be a better marker of glycaemic control and correlate better with retinopathy. However, mean values from Norwich were lower than expected and lower than seen in the 2007 survey of diabetes complications at QECH [1] (mean HbA1c 9.4). There is no obvious seasonal effect: main cohort study visits 1 and 2 occurred during the wet (malaria) season while most of the five year cohort were seen in the dry season. I took the decision to use the measurements from the Norwich laboratory and all samples from visit 3 were therefore tested in Norwich. Possible explanations for the unexpectedly low HbA1c measurements from the MDRS cohort are considered in Chapter 11 of this thesis (Discussion).

Chapter 6. Malawi Diabetic Retinopathy Study: Cross

Sectional Data

6.1 Aims of the chapter

This chapter details the baseline demographic characteristics, systemic variables, prevalence of retinopathy grades and visual acuity data from the Malawi Diabetic Retinopathy Study (MDRS) cohort.

6.2 Introduction

The number of adults with diabetes in Africa is predicted to increase from 12.1 million in 2010 to 23.9 million in 2030 [207]. The prevalence and incidence of sight threatening diabetic retinopathy (STDR) in developed countries are well documented. Associations between systemic factors, including glycaemic control, [12,13,97,314] blood pressure [14,157,314,315,316] and blood lipid levels, [15] and the development and progression of retinopathy in these populations are well known. I conducted with colleagues a systematic review of the literature on the epidemiology of diabetic retinopathy (DR) in Africa which is presented in Chapter 4 [210]. This review identified only 2 cohort studies which investigated determinants of severity and progression: a population based study from Mauritius [212,215] and a relatively small study of people with type 1 diabetes in South Africa [216,217,218]. Neither study was performed in eastern Africa and neither investigated the effect of population specific variables such as HIV infection and anaemia.

A cross sectional survey of 281 persons attending the diabetes clinic at Queen Elizabeth Central Hospital (QECH), Blantyre indicated a prevalence of proliferative retinopathy (PDR) and STDR approximately ten times and six times that seen in Western Europe, respectively [2]. Because of these important findings I set out to estimate the prevalence of grades of DR and visual impairment due to DR in a formal observational study using a systematically sampled cohort, standardised

clinical photography, independent grading by graders in an accredited reading centre and collecting data on covariates specific to the population. The MDRS is a prospective, observational, cohort study of persons attending 2 hospital diabetes clinics over 24 months. The study aims to describe the prevalence, incidence and progression of DR in Southern Malawi and to investigate the determinants of DR severity and progression in this population. In this chapter I report baseline data from this cohort.

6.3 Methods

Study setting, sampling of subjects, clinical assessment and assessment of retinopathy are fully described in Chapter 5 Methods. In brief, subjects were systematically sampled from 2 hospital-based diabetes clinics providing predominantly primary care for diabetes. Visual acuity, glycaemic control, blood pressure, HIV status, urine albumin-creatinine ratio (ACR), haemoglobin and serum lipids were assessed. Retinopathy was graded at an accredited reading centre using modified Wisconsin grading of 4-field mydriatic photographs.

6.3.1 Statistical analysis

An *a priori* analysis plan was followed. Grades of DR were calculated by patient according to the worse or only gradeable eye. Visual acuity data were investigated by patient according to the better eye. 95% confidence intervals (CIs) were calculated for proportions. I constructed a logistic regression model (backwards stepwise with probability of removal of 0.2) to determine the odds ratio (OR) and 95% CIs for the presence of STDR in association with an initial 11 variables: duration of diabetes, age, sex, sBP, glycosylated haemoglobin (HbA1c), urine ACR, haemoglobin level, HIV status, LDL cholesterol, HDL cholesterol and triglycerides. These variables were chosen either because they have been associated with development and progression of DR in other populations (duration, age, sex, sBP, HbA1c, urine ACR, and lipids) or because they are population specific variables, have plausible links to development of DR and whose effect on DR has not been investigated in high quality studies (HIV and anaemia). Adjusted ORs and 95% CIs

were calculated for presence of STDR. All tests were two-sided and considered statistically significant when $p < 0.05$. All calculations were performed using STATA version 12 (StataCorp, Texas, USA).

6.4 Results

6.4.1 Recruitment

417 patients were approached to participate in the study (Figure 6.1 and 6.2). 24 were excluded: 6 did not meet criteria for diagnosis of diabetes; 1 had gestational diabetes; 17 were either under 18 years of age, visiting the clinic for the first time or resident >60km from the clinic. There was an increase in patients with newly diagnosed or suspected diabetes attending the clinic in May 2012. Therefore there was an increase in excluded subjects during this month. 36 declined: 1 had to attend work; 1 was recruited but left before assessment; 34 gave no reason for declining. 357 subjects were included (255 from QECH; 102 from Zomba Central Hospital (ZCH)). Data collection was thorough. The number of subjects with missing data for each variable was as follows: demographic details, HIV and retinopathy grading 0; visual acuity and blood pressure 1 (0.3%); Urine ACR 3 (0.8%), body mass index (BMI) 4 (1.1%); Hb, lipids and serum creatinine 5 (1.4%); HbA1c 6 (1.7%); fasting blood sugar 7 (2.0%).

Figure 6.1. Flow diagram for enrolment of subjects to the MDRS

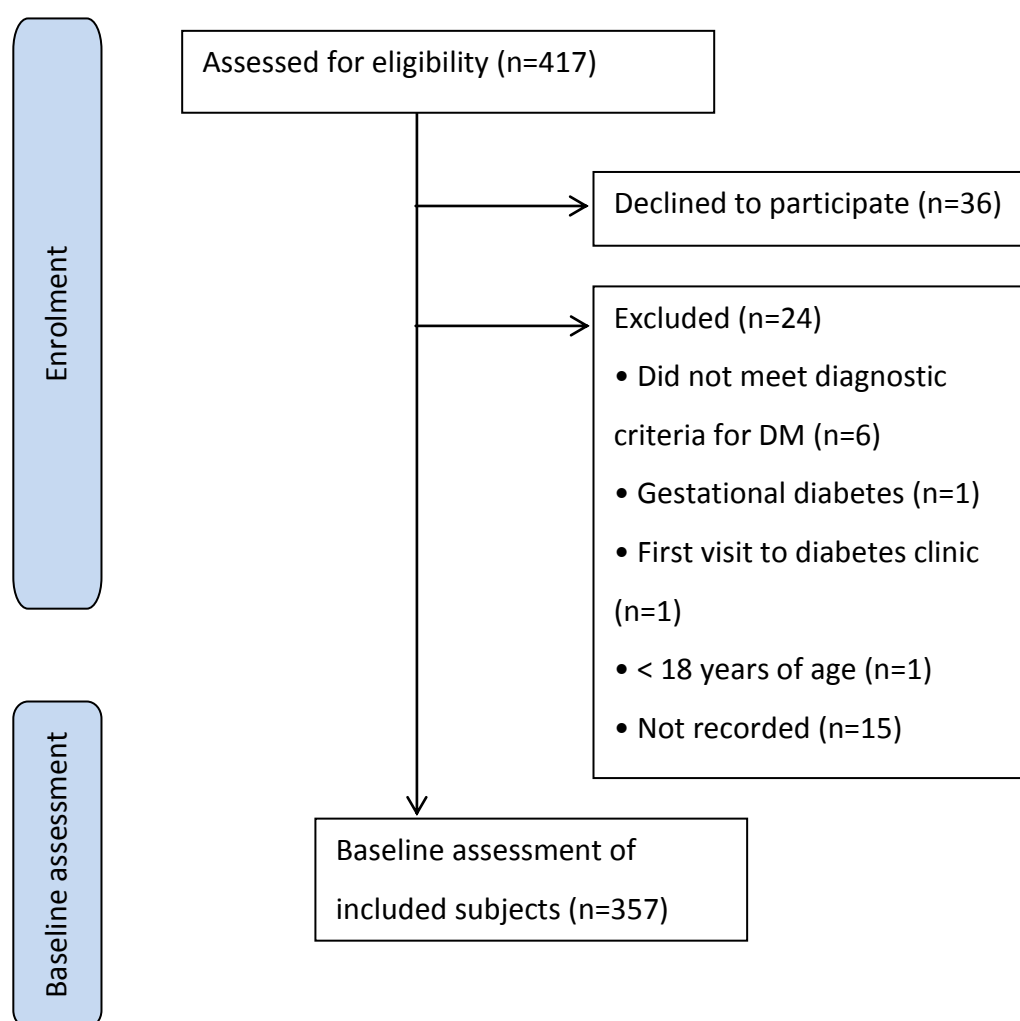
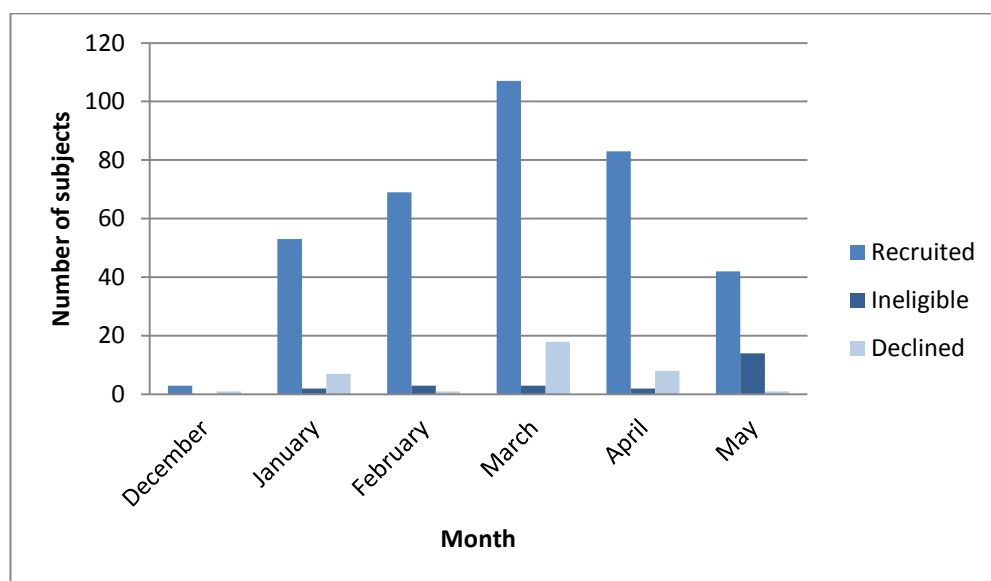


Figure 6.2 Numbers of subjects who were recruited to, excluded from or declined recruitment to the MDRS by month in 2011/12.



6.4.2 Participants

Participant characteristics are listed in Table 6.1. Of those with type 2 diabetes, 231 (71.7%) were prescribed oral agents alone, 12 (3.4%) were diet controlled and 79 (24.5%) were prescribed insulin. 48 (13.4%) subjects were HIV positive: 34 taking anti-retroviral therapy (ART); 4 known HIV+ but not taking ART (all WHO stage 1); and 10 new diagnoses (5 subjects WHO stage 1; 2 stage 2; 2 stage 3; and 1 stage 4). 292 (81.8%) subjects were HIV non-reactive; 17 subjects (4.8%) declined HIV testing. 24 (17.1%) men and 30 women (13.8%) were anaemic as defined above. Of the whole cohort 203 subjects were taking antihypertensive medications. Additionally 31 had either sBP ≥ 140 mmHg or dBP ≥ 90 mmHg (i.e. were newly diagnosed with probable hypertension). 55.3% of subjects had a BMI > 25 kg/m²; Table 6.2 shows study subjects classified according to BMI.

The following non-ocular complications of diabetes were recorded in health passports or reported by subjects: stroke 15 (4.2%); neuropathy 52 (14.5%), foot ulcers 16 (4.5%) of which 8 (2.2%) had amputations; erectile dysfunction 58 (41.1% of men); and ischaemic heart disease 3 (0.8%). 134 (37.5%) subjects reported an episode of malaria in the past 12 months; 23 (6.4%) and 18 (5.0%) reported ever

having TB or syphilis, respectively. 6 subjects (1.7%) were current smokers. 46 (12.9%) of subjects could recall having a dilated eye examination in the past.

Table 6.1 Participant characteristics: demographic, clinical and biochemical measurements of subjects in the MDRS study (n=357)

Characteristic	Entire cohort	Type 1 diabetes	Type 2 diabetes
n	357	35 (9.8%)	322 (90.2%)
Female sex	216 (60.5%)	8 (22.8%)	208 (64.6%)
Age (yrs; median, IQR)	54.1 (43.8-61.1)	28.3 (23.1-33.3)	55.2 (47.9-62.2)
BMI >25 kg/m ²	198 (55.3%)	7 (20.0%)	191 (59.3%)
Duration (yrs; med, IQR)	4.1 (1.9-8.1)	4.1 (1.4-8.2)	4.1 (2.0-8.1)
Hypertensive (see text)	234 (65.5%)	3 (8.6%)	231 (71.7%)
sBP (mmHg; median, IQR)	135 (120-156)	116 (109-127)	138 (124-160)
HbA1c (IFCC, mmol/mol) (mean; SD)	61.9 (27.5)	81.6 (27.8)	59.8 (26.6)
HbA1c (NGSP%; mean; SD)	7.8 (2.5)	9.6 (2.5)	7.6 (2.4)
Haemoglobin (g/dl, mean; SD)	13.9 (1.9)	14.6 (1.8)	13.9 (1.9)
Anaemia (see text)	54 (15.1%)	5 (14.3%)	49 (15.2%)
HIV positive	48 (13.4%)	4 (11.4%)	44 (13.7%)
Total chol. >5.0mmol/L	115 (32.2%)	4 (11.4%)	111 (34.5%)
LDL cholesterol (mmol/L; mean, SD; range)	2.43; 0.95; 0.3- 6.0	1.74; 0.70; 0.6- 3.1	2.51; 0.94; 0.3- 6.0
Raised urine ACR (Male >2.5 mg/mmol; Female >3.5)	115 (32.2%) (51 male; 64 female)	10 (28.6%) (7 male; 3 female)	105 (32.6%) (44 male; 61 female)

Table 6.2 Subjects in the MDRS study classified according to category of BMI (kg/m²)

Category	Range of BMI (kg/m ²)	Number of subjects (%)
Severely underweight	< 16.0	3 (0.8)
Underweight	16.0 \geq x < 18.5	10 (2.8)
Normal	18.5 \geq x < 25	141 (39.5)
Overweight	25 \geq x < 30	126 (35.3)
Obese class 1	30 \geq x < 35	46 (12.9)
Obese class 2	35 \geq x < 40	18 (5.0)
Obese class 3	\geq 40	9 (2.5)
No data		4 (1.1)

6.4.3 Comparison of photographic and clinical DR grading

Biomicroscopy grading was compared with the reference standard of photographic grading. For all grades of retinopathy Cohen's Kappa was 0.6723 (95% CI 0.606-0.738) and weighted Kappa 0.820 (Table 6.3). For grades of maculopathy Cohen's Kappa was 0.843 (95% CI 0.781-0.905) and weighted Kappa 0.888 (Table 6.4). These levels of concordance between photographic and clinical grading are within published acceptable limits for DR grading [317].

Table 6.3 Comparison for all grades of retinopathy between clinical grading and the reference standard of photographic grading (expected frequencies in parentheses) (n=320). Percentage agreement: 78.8%.

		Photographic grade						Total
		10	20	30	40	50	60+	
Clinical grade	10	149 (86.6)	19					168
	20	16	59 (21.6)	3				78
	30		10	11 (1.6)				21
	40		1	7	17 (2.0)		1	26
	50			3	5	5 (0.2)		13
	60+				2	1	11 (0.5)	14
Total		165	89	24	24	6	12	320

Table 6.4 Comparison for grades of maculopathy between clinical grading and the reference standard of photographic grading (expected frequencies in parentheses) (n=320). Percentage agreement: 93.75%.

		Photographic grade							Total
		0	1	2	3	4	8	90	
Clinical grade	0	233 (177.0)				6			239
	1		0 (0)						0
	2	3		3 (0.15)	2	4			12
	3				0 (0.01)	1			1
	4	1		1	1	63 (15.3)			66
	8						1 (0.003)		1
	90		1					0	1
Total		237	1	4	3	74	1	0	320

6.4.4 Prevalence of grades of retinopathy

Prevalence of grades of retinopathy are shown in Table 6.5. Prevalence of retinopathy according to type 1 or type 2 diabetes is shown in Table 6.6. Figure 6.3 shows prevalence of any retinopathy, STDR and PDR categorized by time since diagnosis of diabetes. 87 (24.4%) subjects had cataract (19 unilateral; 68 bilateral). 16 subjects (4.5%) were pseudophakic (5 unilateral; 11 bilateral). There were few subjects with diet controlled diabetes. The Liverpool Diabetic Eye Study (LDES) collected data on subjects with diabetes at point of initiation of a screening programme between 1991 and 1999 [288]. For comparison prevalence of retinopathy grades in persons with type 2 diabetes prescribed oral medications and/or insulin (i.e. excluding diet controlled type 2) from our study and the LDES [288] are shown in Table 6.7.

Table 6.5 Prevalence (95% CI) of retinopathy grades according to worse eye in subjects in the MDRS study (n=357). STDR: Sight threatening diabetic retinopathy.

Grade	n	% (95% CI)
No retinopathy (level 10)	177	49.6 (44.4 - 54.8)
Any retinopathy (level 20-71+)	179	50.1 (44.9 - 55.3)
Level 20 retinopathy	94	26.3 (21.8 – 30.9)
Level 30 retinopathy	25	7.0 (4.4 – 9.7)
Level 40 retinopathy	26	7.3 (4.6 – 10.0)
Level 50 retinopathy	8	2.2 (0.7 – 3.8)
Proliferative or worse (≥ level 60 retinopathy)	26	7.3 (4.6 – 10.0)
Ungradeable	1	0.3 (0 - 0.8)
Sight threatening maculopathy	93	26.1 (21.5 – 30.6)
STDR	105	29.4 (24.7 - 34.1)

Table 6.6 Prevalence with 95% CI of retinopathy grades according to worse eye in persons with type 1 (n=35) and type 2 diabetes (n=322).

	Type 1 diabetes		Type 2 diabetes	
Grade	n	% (95% CI)	n	% (95% CI)
No retinopathy (level 10)	20	57.1 (40.8 – 73.5)	157	48.8 (43.3 – 54.2)
Any retinopathy (level 20-71+)	15	42.9 (26.5 – 59.3)	164	50.9 (45.5 – 56.4)
Level 20	10	28.6 (13.6 - 43.5)	84	26.1 (21.3 – 30.9)
Level 30	1	2.9 (0 – 8.4)	24	7.5 (4.6 – 10.3)
Level 40	1	2.9 (0 – 8.4)	25	7.8 (4.8 – 10.7)
Level 50	0	0	8	2.5 (0.8 – 4.2)
Proliferative or worse (\geq level 60)	3	8.6 (0 – 17.9)	23	7.1 (4.3 – 10.0)
Ungradeable	0	0	1	0.3 (0 – 0.9)
Sight threatening maculopathy	6	17.1 (4.7 – 29.6)	87	27.0 (22.2 – 31.9)
STDR	9	25.7 (11.2 – 40.2)	96	29.8 (24.8 – 34.8)

Figure 6.3. Prevalence (% with 95% CI) of any retinopathy, sight-threatening diabetic retinopathy (STDR) and proliferative diabetic retinopathy (PDR) categorised by time since diagnosis of diabetes in subjects in the MDRS study (n=357)

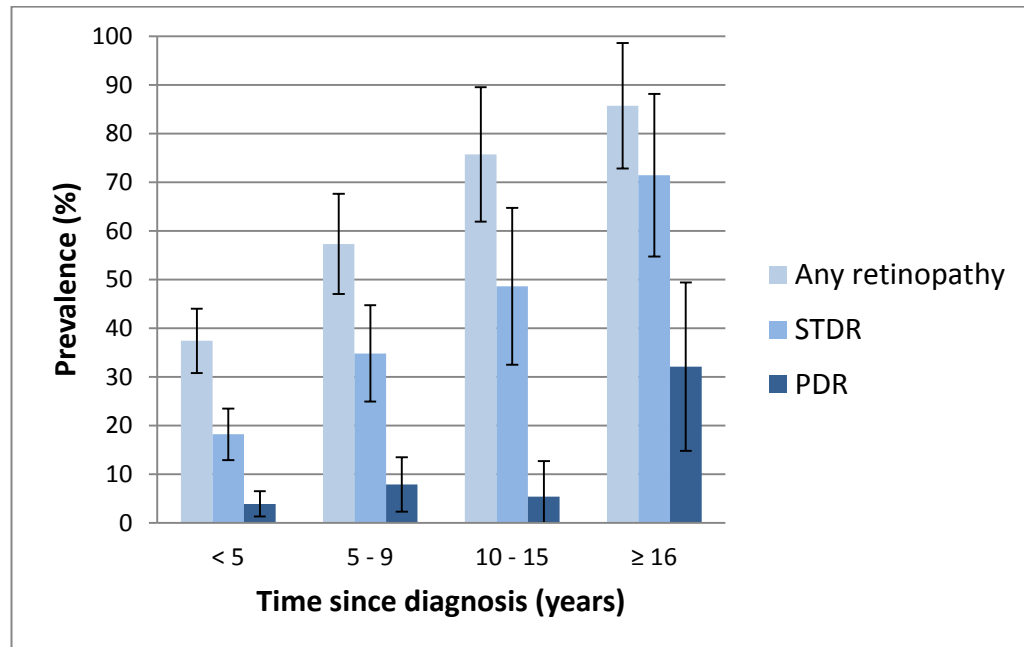


Table 6.7 Prevalence with 95% CI of retinopathy grades according to worse eye in people with type 2 diabetes prescribed oral medications and/or insulin (i.e. excluding diet controlled type 2 diabetes). Comparison of subjects this study (n=310) and the Liverpool Diabetic Eye Study (LDES) (n= 4102).

	MDRS 2011-2012		LDES 1991-1999	
Grade	n	% (95% CI)	n	% (95% CI)
No retinopathy (level 10)	149	48.1 (42.5 – 53.6)	2646	64.5 (63.0-66.0)
Any retinopathy (level 20-71+)	160	51.6 (46.1 – 57.2)	1456	35.5 (34.0-37.0)
Level 20 retinopathy	81	26.1 (21.2 – 31.0)	943	23.0 (21.7 – 24.3)
Level 30 retinopathy	24	7.7 (4.8 – 10.7)	240	5.9 (5.1 - 6.6)
Level 40 retinopathy	24	7.7 (4.8 – 10.7)	163	4.0 (3.4 - 4.6)
Level 50 retinopathy	8	2.6 (0.8 – 4.4)	82	2.0 (1.6 - 2.4)
Proliferative or worse (≥level 60)	23	7.4 (4.5 – 10.3)	28	0.7 (0.4 - 0.9)
Ungradeable	1	0.3 (0 – 1.0)	NA	NA
Sight threatening maculopathy	86	27.7 (22.8 – 32.7)	320	7.8 (7.0 – 8.6)
STDR	94	30.3 (25.2 – 35.4)	348	8.5 (7.6 – 9.3)

6.4.5 Associations of retinopathy

Duration of diabetes, HbA1c, sBP, haemoglobin and LDL cholesterol were risk factors for STDR in multivariate analysis (Table 6.8). Descriptive analysis showed that urine ACR did not demonstrate a linear association with probability of STDR; a natural log transformation was more suitable for the model. There was no difference in prevalence of any retinopathy, sight threatening retinopathy and proliferative retinopathy between subjects from Blantyre and Zomba (data not shown).

Table 6.8 Risk factors for association of presence of sight-threatening diabetic retinopathy (STDR) at baseline in subjects in the MDRS study (n=357).

	OR	95% CI	p value
Univariate logistic regression			
Duration of diabetes (years)	1.13	1.08 – 1.18	0.001*
HbA1c (NGSP %)	1.14	1.04 – 1.25	0.004*
sBP (mmHg)	1.02	1.01 – 1.03	0.001*
log[Urine ACR] (mg/mmol)	1.42	1.22 – 1.65	0.001*
Haemoglobin (g/dl)	0.83	0.73 - 0.94	0.003*
HIV positive	0.43	0.19 – 0.95	0.037*
LDL cholesterol (mmol/L)	1.41	1.10 – 1.80	0.006*
HDL cholesterol (mmol/L)	1.90	0.97 – 3.73	0.060
Triglycerides (mmol/L)	0.99	0.82 – 1.21	0.933
Sex (male)	0.61	0.38 – 0.99	0.045*
Age (years)	1.01	0.99 - 1.03	0.142
Multivariate logistic regression			
Duration of diabetes (years)	1.11	1.05 - 1.17	0.001*
sBP (mmHg)	1.03	1.01 - 1.04	0.001*
HbA1c (NGSP %)	1.31	1.13 - 1.50	0.001*
Haemoglobin (g/dl)	0.80	0.68 - 0.95	0.011*
LDL cholesterol (mmol/L)	1.63	1.18 - 2.25	0.003*
log[Urine ACR] (mg/mmol)	1.19	0.98 - 1.44	0.073
Age (years)	0.97	0.95 - 1.00	0.053

6.4.6 Treatment

One subject had undergone a course of laser photocoagulation prior to study enrolment. 63 subjects were listed for a course of laser treatment at their first study visit. Threshold for scatter laser treatment was the '4-2-1 rule' (4 quadrants of haemorrhages/microaneurysms (HMA) \geq Early Treatment of Diabetic Retinopathy Study (ETDRS) standard photograph 2A, or 2 quadrants of venous beading \geq 6A, or 1 quadrant of intra-retinal microvascular abnormalities (IRMA) \geq 8A). Threshold for macular laser treatment was clinically significant macular oedema (CSMO) or exudates which were tracking towards the centre of the fovea and were therefore 'sight threatening' in the opinion of the examining clinician (PB). Table 6.9 shows the number of subjects who were listed for, started and completed a course of laser treatment.

Table 6.9 Laser treatment of subjects in the MDRS cohort (n=63). Numbers of study subjects listed for laser treatment, started treatment and completed a course of treatment within one year categorised by type of treatment.

Treatment (unilateral or bilateral)	Number of subjects listed for treatment	Started treatment	Completed course
Scatter and macular laser	39	36	29
Scatter alone	11	11	11
Macular laser alone	13	12	12

6.4.7 Vision

Visual acuity measurements for study subjects are shown in table 6.10. According to WHO definitions [310] 343 subjects (96.1 %; 95% CI 94.1 - 98.1) had 'normal vision' (equal to or better than 60 letters), 8 subjects (2.2 %; 95% CI 0.7 - 3.8) had 'moderate visual impairment' (50 to 59 letters), and 5 subjects (1.4 %; 95% CI 0.2-2.6) were 'severely visually impaired or blind' (<50 letters). The most common primary causes of visual impairment, in the opinion of the examining

ophthalmologist (PB), for subjects with corrected visual acuity worse than 80 letters (equivalent to 6/12 Snellen or worse) (n=97) were DR (33.0%), cataract (28.9%), and both DR and cataract (15.5%) (Table 6.11). Therefore in 48.5% of cases DR was the sole or equal contributing cause of visual loss. Figure 6.4 shows visual acuities in the study population classified according to age. In univariate analysis vision < 70 letters was significantly associated with increasing age (years; OR 1.04; 95% CI 1.01 - 1.08; p=0.01), duration of diabetes (years; OR 1.06; 95% CI 1.01 – 1.12; p=0.03) and STDR (OR 2.59; 95%CI 1.16 – 5.79; p=0.02)

Table 6.10 Prevalence with 95% CI of corrected ETDRS visual acuities according to better eye in subjects in the MDRS study (n=357). Approximate Snellen acuities in parentheses.

Visual acuity	n	%	95% CI
≥ 90 (6/5)	88	24.6	20.1 - 29.1
80 - 89 (6/7.5)	171	47.9	42.7 - 53.1
70 - 79 (6/12)	71	19.9	15.8 - 24.0
60 - 69 (6/18)	13	3.6	1.7 – 5.6
50 - 59 (6/30)	8	2.2	0.7 - 3.7
40 - 49 (6/75)	3	0.8	0 - 1.7
Hand movements	1	0.3	0 – 0.8
Light perception	1	0.3	0 - 0.8
No light perception	0	0	
No data	1	0.3	0 – 0.8

Table 6.11 Primary causes of visual impairment in the opinion of the examining clinician (PB) for subjects with corrected visual acuity equivalent to 6/12 Snellen or worse. Subjects classified according to level of visual impairment (n=97).

	Level of visual impairment			
	6/12	6/18	Moderate visual impairment	Severely visually impaired or blind
n	71	13	8	5
DR	24 (34%)	2 (15%)	5	1
DR and cataract	9 (13%)	4 (31%)	1	1
Cataract	20 (28%)	6 (46%)	1	1
AMD	3 (4%)			
Glaucoma	2 (3%)			
Other	13* (18%)	1§ (8%)	1≠	2‡

Details of other causes of visual impairment:

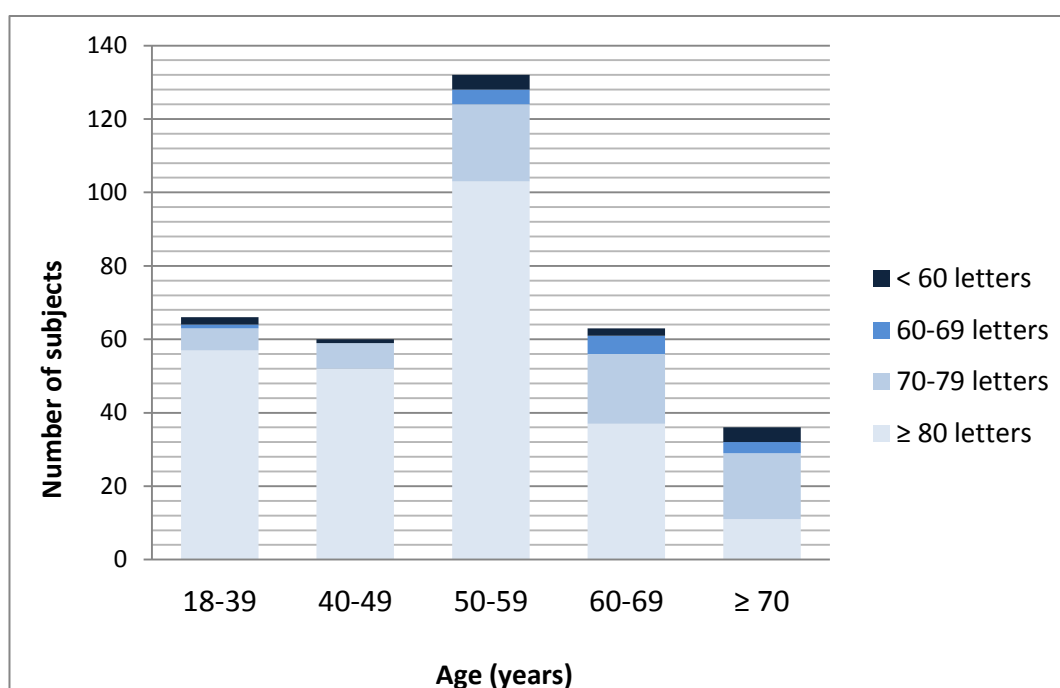
* 1 Posterior capsule opacification (PCO); 2 Epiretinal membrane; 1 optic atrophy; 1 complicated cataract surgery; 1 dry eye; 1 macular hole; 1 unidentified maculopathy; 5 no cause identified.

§ PCO

≠ Optic atrophy secondary to sphenoid meningioma

‡ 1 inherited retinopathy; 1 posterior uveitis (likely syphilitic)

Figure 6.4 Visual acuity in the better eye in subjects in the MDRS study grouped according to age (n=357). Corrected visual acuity classified as ≥ 80 letters, 70-79 letters, 60-69 letters and < 60 letters



6.5 Discussion

6.5.1 Principal findings

This chapter details the baseline prevalence of diabetic retinopathy and visual impairment as well as associations of STDR in the MDRS cohort. Subjects were sampled from a mixed urban and rural population attending for routine primary and secondary diabetes care. Retinopathy was found in 50%; this was sight threatening in 30% with immediately sight-threatening proliferative disease in 7.3%. In multivariate analysis, duration of diabetes, worse glycaemic control, higher systolic blood pressure, lower haemoglobin level and elevated LDL cholesterol were significantly associated with presence of STDR. In this selected population the prevalence of vision in the better eye worse than 70 letters (worse than 6/12) and

60 letters (worse than 6/18) was 7.3% and 3.6%, respectively. In 48.5% of subjects with visual loss DR was the sole or equal contributing cause.

6.5.2 Comparison with African studies

This study found a higher prevalence of any DR, STDR and PDR than reported in the 2007 pilot study from Malawi (any DR 32.0%; STDR 19.6%; PDR 5.7%)[2], which formed part of a larger cross sectional survey of diabetes complications [1]. In that study 281/620 were examined for DR. Higher estimates in my study are likely to reflect differences in subject sampling (systematic vs *ad hoc*), grading of DR (accredited grading of standard photographs vs clinical grading), differences between centres (this study also included persons from Zomba, a more rural setting), and changes in disease prevalence over time.

Two published population based studies from Africa have reported prevalence of DR in persons with diabetes, neither from sub-Saharan Africa (SSA). In these studies from Egypt [239] and Mauritius [212] the prevalence range for any DR was 30.2 to 31.6%, PDR 0.9 to 1.3%, and any maculopathy 1.2 to 4.5%. A recent population based survey (n=4414) from Nakuru, Kenya identified, in 277 persons with diabetes, a prevalence of 'any DR' and 'severe non-proliferative DR or proliferative DR' of 35.9% (95% CI: 29.7-42.6) and 13.9% (10.0-18.8), respectively [A. Bastawrous personal communication, Data submitted for publication].

Clinic-based studies from SSA report a wide range of prevalences but vary widely in quality and methods. Very high prevalence of DR, PDR and maculopathy has been reported in clinic-based surveys from South Africa in the last decade by Mash et al. [241] (62.4% any DR, 6.1% PDR, 15.2% any maculopathy) and Rotchford et al. [224] (40.3% any DR, 5.6% PDR, 10.3% clinically significant macular oedema). These estimates are comparable to the MDRS reflecting similarities between these populations in socioeconomic status, access to health care, diet and levels of infective and non-communicable co-morbidity.

6.5.3 Comparison with studies outside Africa

Population based studies from low and middle income countries have reported lower rates. The Chennai Urban Rural Epidemiology Study (CURES) reported a prevalence of retinopathy of 17.6% in 1736 subjects with type 2 diabetes [318]. In the Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study in urban Indian subjects older than 40 years with diabetes the prevalence of any retinopathy, proliferative retinopathy and CSMO was 18%, 1.6% and 1.4%, respectively [319]. In Europe the population based Liverpool Diabetic Eye Study (LDES) [288] reported findings from 8062 subjects with diabetes (10.3% type 1) entering a primary care-based screening programme. The prevalence of any retinopathy, STDR and PDR was 27.4%, 7.0% and 0.8%, respectively. Other European population-based studies have reported similar prevalence to the LDES [102,285,289-291,301]. The number of subjects with diet controlled diabetes in the MDRS was low. However, even after removing diet controlled subjects from both cohorts, in subjects with type 2 diabetes the prevalence of STDR and PDR in this study compared to the LDES was approximately 3 times and 10 times higher, respectively. The high prevalence of retinopathy in our study compared with recent Asian and Western studies is likely due to late diagnosis of diabetes, poor access to health services, and inadequate drug supply as well as comorbidity.

In common with our study the risk of development and progression of retinopathy in European and North American populations has been shown to be related to duration of diabetes [10,11,314], high HbA1c [12,13,97,314], high blood pressure [14,157,314-316], microalbuminuria [320] and serum lipid levels [15]. Associations between retinopathy and age [10] and gender [10] have been demonstrated but were not apparent in our cohort. Prevalence of proteinuria and hypercholesterolemia in this study are similar to previous cross sectional work from the Blantyre diabetes clinic [1,321] detailed in Chapter 4, Section 4.7.2. The median time since diagnosis of diabetes in our cohort is relatively short at 4 years. Although very likely to be an underestimate of disease duration, this makes the high prevalence of STDR even more striking. The mean HbA1c in our cohort (7.8%) is surprisingly low. The 2007 Blantyre study of diabetic complications reported a

mean of 9.4%. [1] Since 2007 several care improvement initiatives have been implemented in the diabetes clinics at QECH and ZCH [322]. In addition compared with the current cohort the 2007 subjects included a higher proportion of type 1 subjects (18%), who seem to have worse control, a higher proportion of type 2 subjects taking insulin (29%) and a longer mean duration of diabetes (7.0 yrs).

6.5.4 Associations of STDR

This study has demonstrated an association between lower haemoglobin level and presence of STDR. Cross sectional (but not cohort) studies have demonstrated an association between presence of DR and anaemia in India [323-325] and China [187]. To my knowledge this relationship has not been shown previously in an African population. We hypothesise that the mechanism underlying this relationship is impaired oxygen delivery and therefore increased oxygen stress at a microvascular level. The aetiology of anaemia in SSA is multifactorial and includes deficiencies of micronutrients (e.g. iron, B12, folate); haemoglobinopathies; infections and chronic diseases (e.g., malaria, HIV, tuberculosis)[183]. Micronutrient deficiencies are potential therapeutic targets. Whether treatment of anaemia reduces diabetic microvascular complications is not known. A potential confounder of the association between haemoglobin and retinopathy is socioeconomic status. Socioeconomic data was not collected in this study.

Both HIV infection and anti-retroviral therapies are associated with a vasculopathy which manifests as increased cardiovascular and cerebrovascular risk [326,327]. There is also evidence of a higher prevalence of diabetic microvascular complications in persons with HIV [1]. This study showed no significant relationship in multivariate analysis between presence of STDR and HIV status. In univariate analysis a protective effect of HIV is suggested. We believe that early diagnosis of diabetes in this subgroup is an important confounder: patients attending medical facilities for ART treatment are more likely to be tested for diabetes than the general population. The effect of HIV status on DR progression will be shown by the MDRS cohort study.

6.5.5 Vision

Few studies have investigated VA in people with diabetes in SSA. Prevalence of visual impairment in this study (1.4% of subjects with VA 6/60 or worse in the better eye) is comparable with published European and American data. In the Wisconsin Epidemiological Study of DR (WESDR) a VA of 6/60 (US equivalent 20/200) or worse in the better eye occurred in 3.6% of type 1 subjects and 1.6% of type 2 subjects [328]. In Iceland, Kristinsson et al. [290] reported VA of 6/60 or worse in the better eye in 1.0% of type 1 subjects and 1.6% of type 2 subjects. The similar levels of visual impairment are surprising given the higher prevalence of STDR in our cohort. A potential bias is that subjects who become visually impaired may cease to attend clinics. Visual impairment may significantly increase chance of mortality in a society where loss of vision entails loss of economic productivity. Supporting this possible explanation are our results showing a high proportion of people attending clinics with STDR which is not yet symptomatic. This disease is potentially treatable to prevent visual loss; the case for intervention is then extremely strong. In this study 63 subjects were listed for a course of laser treatment while only 1 patient had received laser treatment prior to the study. This equates to a laser coverage of 1.6% at the time of the study.

6.5.6 Limitations of this work

These findings are likely to be representative of small cities/large towns in SSA but should be generalized to other settings with some caution. While some patients travel long distances to attend clinics, rural subjects are likely to be underrepresented and form a selected sub-group of the rural diabetes population. A significant proportion of patients spend some time in the city and some (e.g. at planting and harvesting time) at their village; differentiating rural and urban populations is difficult. It is possible that our data underestimate retinopathy. Patients who do not attend clinics may be less likely to be diagnosed with diabetes or to comply with therapy. Conversely those with established complications may be more likely to attend clinics and participate in research studies. Despite these

limitations, I believe that the size of this study and the degree of confidence around my findings render them useful.

6.6 Chapter summary

The results presented in this chapter provide an estimate of current prevalence of DR and visual impairment in a mixed urban and rural population attending diabetes clinics in Southern Malawi. I have demonstrated a novel association of STDR: haemoglobin level, a population specific target for intervention. I have reported the number of subjects requiring laser treatment. The prevalence of diabetes in Africa is increasing rapidly and there is an urgent need for service provision. This study provides data which is vital for the design of prevention and early detection programmes in the region which I will address further in this thesis. These findings represent a baseline against which the efficacy and cost-effectiveness of such interventions can be judged.

Chapter 7. 12 month Follow-up of the Malawi Diabetic Retinopathy Study Cohort

7.1 Aims of the chapter

This chapter details the demographic characteristics, clinical and biochemical parameters, progression of retinopathy grades and visual acuity data for the Malawi Diabetic Retinopathy Study (MDRS) cohort at 12 month follow-up.

7.2 Introduction

The MDRS is a prospective, observational, cohort study of persons attending 2 hospital diabetes clinics over 24 months. The study aims to describe the prevalence, incidence and progression of diabetic retinopathy (DR) in Southern Malawi and to investigate the determinants of DR severity and progression in this population. The MDRS uses a systematically sampled cohort, standardised clinical photography, independent grading by graders in an accredited reading centre and collects data on covariates specific to the population. In this chapter I report a planned interim analysis from this cohort 12 months after the baseline assessment. As this is an interim analysis and in order to avoid repetition, a detailed discussion of the findings is covered in Chapter 8.

7.3 Methods

7.3.1 Setting, subjects and clinical assessment

Study setting, sampling of subjects, clinical assessment and assessment of retinopathy are described in Chapter 5 methods. Subjects were systematically sampled from 2 hospital based, primary care diabetes clinics. At 12 months, visual acuity, glycaemic control (HbA1c), blood pressure, HIV status, urine albumin creatinine ratio (ACR) and haemoglobin were assessed. Retinopathy was graded at

an accredited reading centre using modified Wisconsin grading of 4-field mydriatic photographs.

7.3.2 Subject tracing

Tracing of subjects was systematic. All subjects recruited to MDRS main cohort were asked to give telephone numbers and an address. Telephone was the first line method of contact. If a subject could not be contacted by telephone they received a home visit from the MDRS study team. Additionally the MDRS study team attended the diabetes clinic weekly between December 2012 and May 2013 to approach patients in the clinic waiting room. Finally the president and vice president of the patient's organisation the Diabetes Association of Malawi reviewed the list of subjects in order to personally identify their whereabouts.

7.3.3 Confirmation of subject death

Confirmation of subject death was performed in a systematic manner. The relatives of subjects reported to be deceased were visited at home by the study research nurse (Chrissy Pindani) between December 2012 and May 2013. The nurse was trained to record, on a standard form, brief written narratives from families or other reliable informants. If available, the death certificate and the health passport were reviewed and cause of death and/or brief details of last illness recorded. A subject was recorded as dead if confirmed by a relative or 'Traditional Authority' (village leader in rural districts), or if a death certificate or marked grave was seen by the study nurse. I assigned a probable cause of death after reading the form.

7.3.4 Statistical analysis

An *a priori* analysis plan was followed. The MDRS was powered for progression of DR at 24 months. However, an interim analysis was scheduled at 12 months. As described in Chapter 5 Methods, grades of DR were calculated by patient according to the worse or only gradeable eye. Visual acuity (VA) data were investigated by patient according to the better eye. 95% confidence intervals (CIs) were calculated for proportions. I constructed a logistic regression model (backwards stepwise with

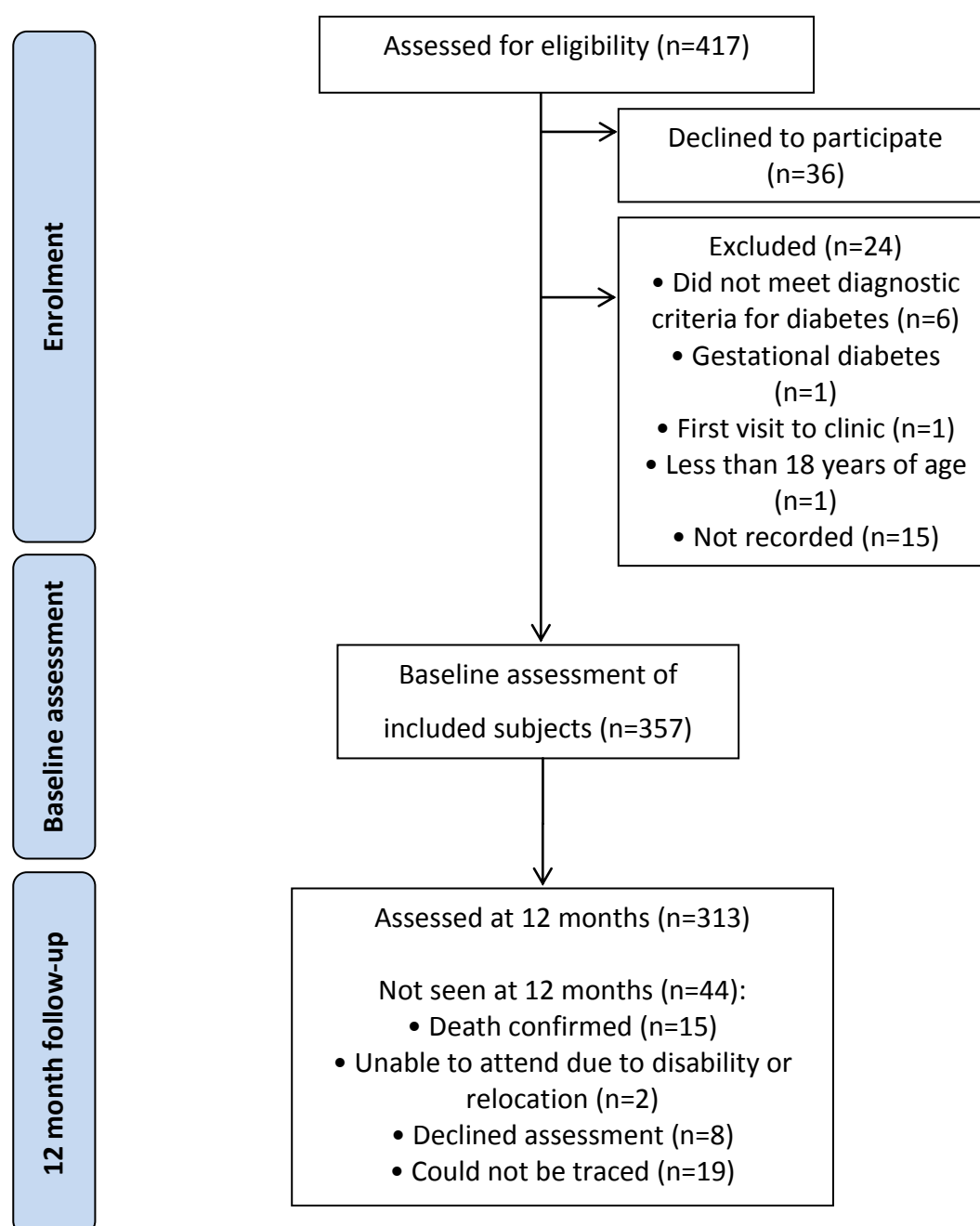
probability of removal of 0.2) to determine the odds ratio (OR) and 95% CIs for 2 step progression on the Liverpool Diabetic Eye Study (LDES) scale in association with an initial 10 variables: time since diagnosis of diabetes, baseline grade of DR, mean HbA1c (mean of visits 1 and 2), mean sBP, mean urine ACR, mean haemoglobin, triglycerides (baseline measurement), HIV status, age, and scatter laser treatment (anytime between visit 1 and visit 2). Adjusted odds ratios (OR) and 95% CIs were calculated for 2 step retinopathy progression.

7.4 Results

7.4.1 Participants

A total of 313 subjects were assessed between December 2012 and May 2013 (Figure 7.1). The death of 15 subjects was confirmed (total 92% follow-up of the original 357 subject cohort). 8 subjects were traced but declined assessment. 1 subject had moved away from Southern Malawi and was unable to return for assessment. 1 subject was unable to attend due to disability. 19 subjects could not be traced.

Figure 7.1 Flow diagram for subjects in the MDRS: enrolment and follow-up at 12 months



7.4.2 Subjects confirmed deceased

15 subjects were confirmed deceased by the MDRS study team by May 2013.

Baseline characteristics and grades of retinopathy of these subjects are shown in Table 7.1 and 7.2. Three death certificates were available. Causes of death recorded were 'upper gastrointestinal bleeding', 'meningitis' and 'diabetes and ascites'. Three health books were reviewed. Causes of death recorded were 'malaria/hypoglycaemia', 'diabetic ketoacidosis' and 'hypoglycaemia/anaemia'. For 9 subjects the cause of death was assigned based on verbal reports alone: 'diabetic ketoacidosis' 1 subject, 'hypoglycaemia' 1 subject, 'renal failure secondary to diabetes' 1 subject, 'Anaemia' 1 subject and 'unknown cause' 5 subjects.

Table 7.1 Baseline characteristics of 15 subjects from the MDRS cohort study confirmed dead by the MDRS team by May 2013. BMI = body mass index. ACR = albumin creatinine ratio.

Characteristic	Level
Female sex	7 (47%)
Age (median, IQR)	56.2 yrs (51.4 – 63.7)
Type 1 diabetes	2
BMI (mean, SD)	23.3 kg/m ² (4.6)
Overweight (BMI>25 kg/m ²)	5 (33%)
Time since diagnosis of diabetes (median, IQR)	8.7 yrs (4.9 – 13.9)
Hypertensive (see text)	8 (53%)
sBP (median, IQR)	130 mmHg (117-152)
dBp (median, IQR)	79 mmHg (71.5 - 89)
Mean arterial pressure (median, IQR)	96 mmHg (89-110)
HbA1c (NSGP) (mean, SD)	8.1% (2.6)
Fasting blood sugar (mean, SD)	15.1 mg/dL (11.8)
HIV reactive	5 (33%)
Anaemia (WHO definition)	9 (60%) 6M; 3F
Total cholesterol >5.0mmol/L	2 (13%)
Total cholesterol (mmol/L; mean, SD)	3.4 (1.3)
HDL cholesterol (mmol/L; mean, SD)	0.90 (0.39)
LDL cholesterol (mmol/L; mean, SD)	1.75 (0.94)
Triglycerides (mmol/L; mean, SD)	1.34 (0.71)
Urine ACR raised (n; %) (M>2.5/F>3.5mg/mmol)	12 (80%)
Serum creatinine (mean, SD)	152 µmol/L (160)
Raised serum creatinine (M>110; F>90 µmol/l)	6 (40%)

Table 7.2 Baseline prevalence of grades of retinopathy for 15 subjects from the MDRS cohort study confirmed dead by the MDRS team by May 2013. STDR = sight threatening retinopathy.

Grade	n
No retinopathy (level 10)	5 (33%)
Any retinopathy (level 20-71+)	10 (66%)
Level 20 retinopathy	2 (13%)
Level 30 retinopathy	3 (20%)
Level 40 retinopathy	1 (7%)
Level 50 retinopathy	0
Proliferative or worse (\geq level 60)	4 (27%)
Ungradeable	0
Sight threatening maculopathy	5 (33%)
STDR	8 (53%)
No data	0

7.4.3 Analysis of bias

In order to determine the degree to which loss to follow-up may bias results of this cohort study, baseline data from subjects seen at 12 months and those lost to follow-up were compared. Baseline demographic, clinical and biochemical parameters of the 357 subjects in the MDRS cohort categorised by follow-up are shown in Table 7.3. There was no significant difference between subjects seen at 12 months and those not seen at 12 months regarding mean duration of diabetes, HbA1c, sBP, dBP, mean arterial pressure (MAP), BMI, triglycerides, and mean total, HDL and LDL cholesterol. There was no significant difference between the proportions of subjects in each group who were hypertensive, overweight, HIV positive, of female sex, had type 1 diabetes or who had raised cholesterol or raised urine ACR.

Those subjects not seen at 12 months demonstrated higher mean age, higher fasting blood sugar, lower haemoglobin and higher serum creatinine than subjects

seen at 12 months. A greater proportion of subjects not seen at 12 months were anaemic and had raised creatinine. Baseline prevalence of grades of retinopathy for 357 subjects in the MDRS cohort categorised by follow-up are shown in Table 7.4. There was no significant difference between subjects seen at 12 months and not seen at 12 months regarding prevalence of any DR, sight threatening retinopathy (STDR) and proliferative diabetic retinopathy (PDR). Table 7.5 shows baseline prevalence of corrected Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuities according to better eye for 357 subjects in the MDRS cohort categorised by follow-up. Subjects who were not seen at 12 months had worse visual acuities than those seen at 12 months ($p=0.003$, χ^2 test for trend).

Table 7.3 Baseline demographic, clinical and biochemical parameters of 357 subjects in the MDRS cohort categorised by follow-up: traced and assessed at 12 months (n=313) or not seen at 12 months (n=44). MAP = mean arterial pressure; FBS = fasting blood sugar.

Characteristic at baseline	Subjects seen at 12 months	Subjects not seen at 12 mths	p value
n	313	44	NA
Female sex	188 (60.1%)	28 (63.6%)	p=0.74 Fisher's exact
Age (yrs; median, IQR)	53.5 (42.5-60.3)	57.4 (53.2-65.7)	p=0.005* Unp'd t-test
Type 1 diabetes	32 (10.2%)	3 (6.8%)	p=0.60 Fisher's exact
BMI (kg/m ² ; mean, SD)	26.5 (5.7)	25.0 (5.6)	p=0.10 Unp'd t-test
BMI >25 kg/m ²	175 (55.9%)	23 (52.3%)	p=0.75 Fisher's exact
Duration (median, IQR)	4.1 (2.0 – 7.7)	4.9 (1.7 – 9.3)	p=0.40 Wilcoxon r. sum
Hypertensive (see text)	207 (66.1%)	27 (61.4%)	p=0.61 Fisher's exact
sBP (mmHg; median, IQR)	135 (120 - 156)	134 (119 - 161)	p=0.89 Unpaired t-test
dBp (mmHg; median, IQR)	82 (74-91)	79 (74 - 89)	p=0.64 Unpaired t-test
MAP (mmHg; median, IQR)	100 (90 - 112)	97 (89 – 115)	p=0.97 Unpaired t-test
HbA1c (NGSP %; mean, SD)	7.8 (2.4)	7.9 (3.2)	p=0.81 Unpaired t-test
FBS (mg/dL; mean, SD)	10.1 (5.9)	12.4 (9.0)	p=0.025* Unp'd t-test
Hb (g/dl) (mean; SD)	14.1 (1.8)	12.6 (2.0)	p=0.0001* Unp'd t-test
Anaemia (see text)	40 (12.8%)	14 (31.8%)	p=0.003* Fisher's exact
HIV positive	41 (13.1%)	7 (15.9%)	p=0.64 Fisher's exact
Total chol. >5.0mmol/L	94 (30%)	11 (25%)	p=0.60 Fisher's exact
Total chol (mmol/L;mean,SD)	4.35 (1.25)	3.97 (1.42)	p=0.064 Unpaired t-test
LDL chol. (mmol/L;mean,SD)	2.47 (0.93)	2.19 (1.06)	p=0.067 Unp'd t-test
HDL chol (mmol/L;mean,SD)	0.99 (0.33)	0.98 (0.41)	p=0.86 Unpaired t-test
Triglycerides(mmol/L;mean,SD)	1.57 (1.22)	1.24 (0.76)	p=0.082 Unpaired t-test
Raised uACR ‡	101 (32.3%)	21 (47.7%)	p=0.061 Fisher's exact
Serum creat (µmol/L;mean,SD)	62.0 (23.0)	90.7 (102.9)	p=0.0001* Un-p'd t-test
Raised creatinine	14 (4.5%)	6 (13.6%)	p=0.025* Fisher's exact

‡Raised urine ACR: Male>2.5mg/mmol; Female>3.5mg/mmol. † Raised serum creatinine: Male>110 µmol/l; Female >90µmol/l

Table 7.4 Baseline prevalence of grades of retinopathy of 357 subjects in the MDRS cohort categorised by follow-up: traced and assessed at 12 months (n=313) or not seen at 12 months (n=44). ST= sight threatening.

Grade (n; %; 95% CI)	Subjects seen at 12 months	Subjects not seen at 12 months	p value (Fisher's exact)
n	313	44	
No DR (level 10)	155 (49.5; 44.0-55.0)	22 (50.0; 35.2-64.8)	p = 0.99
Any DR (level 20-71+)	157 (50.2; 44.7-55.7)	22 (50; 35.2-64.8)	p = 0.99
Level 20 retinopathy	85 (27.2; 22.3-32.1)	9 (20.5; 8.6-32.4)	
Level 30 retinopathy	21 (6.7; 3.9-9.5)	4 (9.1; 0.6-17.6)	
Level 40 retinopathy	22 (7.0; 4.2-9.8)	4 (9.1; 0.6-17.6)	
Level 50 retinopathy	8 (2.6; 0.8-4.4)	0	
Proliferative (≥60)	21 (6.7; 3.9-9.5)	5 (11.3; 2.0-20.7)	p = 0.35
Ungradeable	1 (0.3; 0-0.9)	0	
ST maculopathy	84 (26.8; 21.9-31.7)	9 (20.5; 8.6-32.4)	
STDR	91 (29.1; 24.1-34.1)	14 (31.8; 18.0-45.6)	p = 0.73
No data	0	0	

Table 7.5 Baseline prevalence of corrected visual acuities according to better eye (ETDRS letters) for 357 subjects in the MDRS cohort categorised by follow-up: traced and assessed at 12 months (n=313) or not seen at 12 months (n=44). Approximate Snellen acuities in parentheses.

Visual acuity	313 subjects seen at 12 months		44 subjects not seen at 12 months	
	n	% (95% CI)	n	% (95% CI)
≥ 90 (6/5)	86	27.5 (22.6-32.5)	2	4.5 (0-10.6)
80 - 89 (6/7.5)	148	47.3 (41.8-52.8)	23	52.2 (37.4-67.0)
70 - 79 (6/12)	60	19.2 (14.8-23.6)	11	25.0 (12.2-37.8)
60 - 69 (6/18)	8	2.6 (0.8-4.4)	5	11.4 (2.0-20.8)
50 - 59 (6/30)	7	2.2 (0.6-3.8)	1	2.3 (0-6.7)
40 - 49 (6/75)	2	0.6 (0-1.5)	1	2.3 (0-6.7)
Hand Movements	1	0.3 (0-0.9)	0	
Light Perception	1	0.3 (0-0.9)	0	
No light perception	0		0	
No data	0		1	2.3 (0-6.7)

7.4.4 Demographics and clinical and biochemical measurements of subjects seen at 12 months

For 313 subjects seen at month 12 (visit 2), median time to follow-up was 0.90 years (range 0.80 – 1.25). At baseline 41 subjects (13.1%) were HIV positive. At 12 months an additional 2 subjects were HIV reactive (1 subject WHO stage 1 and 1 subject who had already commenced anti-retroviral therapy (ART)). 6 subjects (1.9%) declined testing. No trend toward worsening renal function was identified: Table 7.6 shows urine ACR measurements at visit 1 and 2.

Table 7.6 Urine albumin creatinine ratio (ACR) measurements for 313 subjects in the MDRS seen at visit 1 and visit 2 (12 months)

Characteristic	Visit 1 (baseline)	Visit 2 (12 months)
Urine ACR (mg/mmol; mean; SD)	13.6 (65.4)	9.5 (30.2)
Urine ACR raised (n; %) (M>2.5;F>3.5 mg/mmol)	101 (32.3%) (43 men; 58 women)	92 (29.4%) (36 men; 56 women)
Urine ACR > 30 mg/mmol (n; %)	21 (6.7%) (5 men; 16 women)	18 (5.8%) (4 men; 14 women)

7.4.5 Prevalence of grades of retinopathy

Prevalence of grades of retinopathy for 313 subjects seen at visit 1 and visit 2 are shown in Table 7.7.

Table 7.7 Prevalence with 95% CI of retinopathy grades according to worse eye in 313 subjects in the MDRS seen at visit 1 and 2.

Grade (n; %; 95% CI)	Visit 1 (baseline)	Visit 2 (12 months)
No retinopathy (level 10)	155 (49.5; 44.0-55.0)	155 (49.5; 44.0-55.0)
Any retinopathy (level 20-71+)	157 (50.2; 44.7-55.7)	156 (49.8; 44.3-55.3)
Level 20 retinopathy	85 (27.2; 22.3-32.1)	73 (23.3; 18.6-28.0)
Level 30 retinopathy	21 (6.7; 3.9-9.5)	36 (11.5; 8.0-15.0)
Level 40 retinopathy	22 (7.0; 4.2-9.8)	20 (6.4; 3.7-9.1)
Level 50 retinopathy	8 (2.6; 0.8-4.4)	4 (1.3; 0.1-2.6)
Proliferative or worse (≥60)	21 (6.7; 3.9-9.5)	23 (7.3; 4.4-10.2)
Ungradeable	1 (0.3; 0-0.9)	2 (0.6; 0-1.5)
Sight threatening maculopathy	84 (26.8; 21.9-31.7)	83 (26.5; 21.6-31.4)
STDR	91 (29.1; 24.1-34.1)	92 (29.4; 24.4-34.5)
No data	0	0

7.4.6 Treatment

Of 313 subjects seen at 12 months two were classified as ungradeable (neither received laser treatment). Therefore data for 2 visits was available for 311 subjects. Of these subjects, within the first year of the study 34 were listed for both scatter laser photocoagulation and macular laser treatment (either unilaterally or bilaterally); 31 started the course of treatment and 25 completed the course. 9 were listed for scatter laser alone; all completed the course. 12 were listed for macular laser treatment alone; all completed the course. Table 7.8 shows laser treatment within the first year of the MDRS (i.e. before visit 2) for subjects classified by baseline level of retinopathy.

Table 7.8 Laser treatment within the first year of the MDRS study for 311 subjects seen at baseline and visit 2 (12 months). Subjects classified by baseline level of retinopathy.

Baseline level of retinopathy	n	Laser photocoagulation (listed / started / completed course)		
		Scatter and macula	Scatter alone	Macula alone
Level 10	154	0	0	0
Level 20	85	0	0	6/6/6
Level 30	21	2/1/1	2/2/2	4/4/4
Level 40	22	10/8/7	1/1/1	2/2/2
Level 50	8	6/6/4	2/2/2	0
Level 60	13	10/10/8	2/2/2	0
Level 70+	8	6/6/5	2/2/2	0
Total	311	34/31/25	9/9/9	12/12/12

7.4.7 Progression of grades of retinopathy

Incidences of development of grades of retinopathy for 311 subjects with level 10 (no retinopathy), level 20, 30, 40, 50, 60 and level 70+ retinopathy at baseline are shown in Tables 7.9 to 7.15, respectively. Incidence of STDR ($p<0.001$), ST maculopathy ($p<0.001$) and PDR ($p<0.001$) increased with severity of baseline

retinopathy (X^2 test for trend). Two (or more) step progression was observed in 29 subjects (9.3%; 95% CI 6.1-12.5); three (or more) step progression in 11 subjects (5.3%; 2.8-7.8). Of 220 subjects without STDR at baseline (visit 1) 10 (4.5%; 1.8-7.2) had developed the condition at visit 2. Of 225 subjects without ST maculopathy at baseline (and whose maculopathy was gradeable) 11 (4.9%; 2.1-7.7) developed the condition by visit 2.

Of 269 subjects not listed for scatter laser at baseline 11 (4.1%; 1.7-6.5) developed retinopathy requiring scatter laser by visit 2. Of 268 subjects not listed for macular laser at baseline 13 (4.9%; 2.3-7.5) developed maculopathy requiring macular laser by visit 2. 4 subjects developed retinopathy requiring both scatter and macular laser. Figure 7.2 shows incidence of progression to level 60+ (PDR) and of 2 step and 3-step progression for subjects with level 10, 20, 30, 40 and 50 retinopathy at baseline.

Table 7.9 Incidence of development of all grades of DR, any DR, sight threatening (ST) maculopathy, STDR, 2-step progression and 3-step progression for 154 persons with level 10 (no retinopathy) at baseline.

Grade progression	Number entering time interval	n	Incidence % (95% CI)
10 - 10	154	136	88.3 (83.2-93.4)
10 - 20	154	12	7.8 (3.6-12.0)
10 - 30	154	6	3.9 (0.8-7.0)
10 - 40+	154	0	0
10 - 20+ (any DR)	154	18	11.7 (6.6-16.8)
10 - ST maculopathy	154	0	0
10 - STDR	152	0	0
10 - 2 step (or greater) progression	154	8	5.2 (1.7-8.7)
10 - 3 step (or greater) progression	154	2	1.3 (0-3.1)

n = number of subjects reaching endpoint

Table 7.10 Incidence of development of all grades of DR, sight threatening (ST) maculopathy, STDR, 2-step progression and 3-step progression for 85 persons with level 20 retinopathy at baseline.

Grade progression	Number entering time interval	n	Incidence % (95% CI)
20 - 10	85	19	22.4 (13.5-31.3)
20 - 20	85	52	61.2 (50.8-71.6)
20 - 30	85	12	14.1 (6.7-21.5)
20 - 40	85	2	2.4 (0-5.6)
20 – 50+	85	0	0
20 - ST maculopathy	63	7	10.8 (3.1-18.4)
20 – STDR	63	9	14.3 (5.7-22.9)
20 – 2 step (or greater) progression	85	6	7.1 (1.6-12.5)
20 - 3 step (or greater) progression	85	2	2.4 (0-5.6)

n =number of subjects reaching endpoint

Table 7.11 Incidence of development of all grades of DR, sight threatening (ST) maculopathy, STDR, 2-step progression and 3-step progression for 21 persons with level 30 retinopathy at baseline.

Grade progression	Number entering time interval	n	Incidence %
30 - 20	21	4	19.0 (2.3-35.8)
30 - 30	21	11	52.4 (31.0-73.7)
30 - 40	21	6	28.6 (9.2-47.9)
30 – 50+	21	0	0
30 - ST maculopathy	4	0	0
30 – STDR	4	0	0
30 - 2 step (or greater) progression	21	5	23.8 (5.6-42.0)
30 - 3 step (or greater) progression	21	1	4.8 (0-13.9)

n =number of subjects reaching endpoint

Table 7.12 Incidence of development of level 20 or 30 retinopathy, level 40, level 50, proliferative DR, ST maculopathy, 2-step progression and 3-step progression for 22 persons with level 40 retinopathy at baseline.

Grade progression	Number entering time interval	n	Incidence %
40 – 20/30	22	8	36.4
40 - 40	22	9	40.9
40 - 50	22	1	4.5
40 - 60+ (proliferative DR)	22	4	18.2
40 - ST maculopathy	3	3	100
40 - 2 step (or greater) progression	22	4	18.2
40 - 3 step (or greater) progression	22	4	18.2

n =number of subjects reaching endpoint

Table 7.13 Incidence of development of retinopathy less than level 50, level 50, proliferative DR, ST maculopathy, 2-step progression and 3-step progression for 8 persons with level 50 retinopathy at baseline.

Grade progression	Number entering time interval	n	Incidence %
50 – <50	8	4	50
50 - 50	8	1	12.5
50 - 60+ (proliferative DR)	8	3	37.5
50 - ST maculopathy	1	0	0
50 - 2 step (or greater) progression	8	2	25
50 - 3 step (or greater) progression	8	1	12.5

n =number of subjects reaching endpoint

Table 7.14 Incidence of development of retinopathy less than level 60, level 60, ST maculopathy, 2-step progression and 3-step progression for 13 persons with level 60 retinopathy at baseline.

Grade progression	Number entering time interval	n	Incidence %
60 – <60	13	5	38.5
60 - 60	13	6	46.1
60 - >60	13	2	15.4
60+ - ST maculopathy	1	1	100
60 - 2 step (or greater) progression	13	1	7.7
60 - 3 step (or greater) progression	13	1	7.7

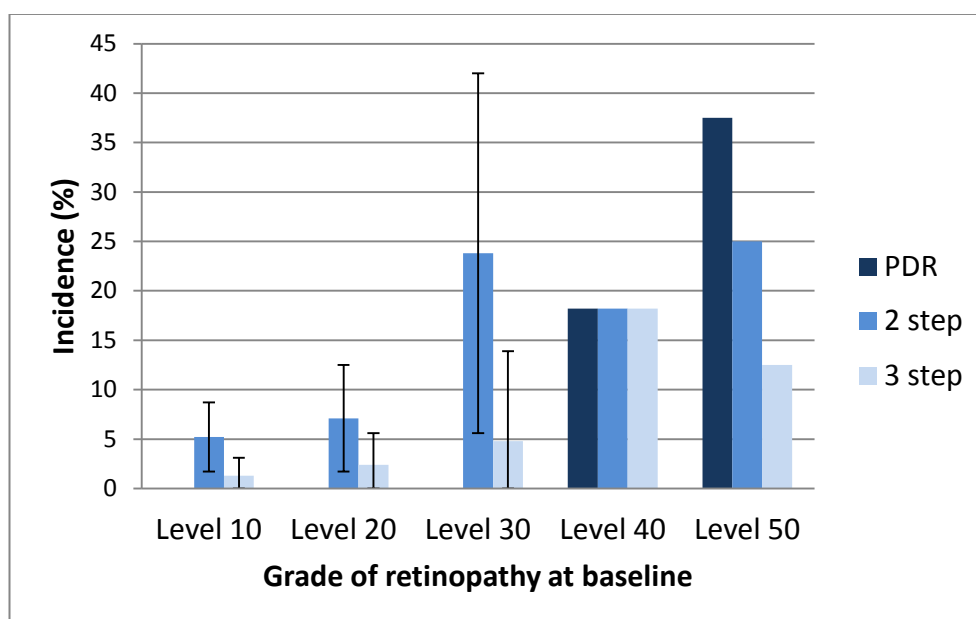
n =number of subjects reaching endpoint

Table 7.15 Incidence of development of level 60 retinopathy, 2-step progression and 3-step progression for 8 persons with level 70+ retinopathy at baseline.

Grade progression	Number entering time interval	n	Incidence %
70+ – 60	8	1	12.5
70+ - 70+	8	7	87.5
70+ - 2 step (or greater) progression	8	3	37.5
70+ - 3 step (or greater) progression	8	0	0

n =number of subjects reaching endpoint

Figure 7.2 Incidence of progression to proliferative diabetic retinopathy (PDR; level 60+) and of 2 step and 3-step progression for subjects with level 10 (n=154), level 20 (n=85), level 30 (n=21), level 40 (n=22) and level 50 (n=8) retinopathy at baseline. Error bars indicate 95% CI. Error bars not shown for level 40 and 50 due to the small number of subjects in these groups.



7.4.8 Associations of progression of retinopathy

Mean HbA1c was a risk factor for 2 step progression on the LDES scale in multivariate analysis (Table 7.16). Descriptive analysis indicated that urine ACR did not demonstrate a linear association with probability of 2 step progression; a natural log transformation was more suitable for the model.

Table 7.16 Risk factors for association of progression of diabetic retinopathy by 2 or more steps on the LDES scale at 12 months in the MDRS (n=311).

	OR	95% CI	p value
Univariate logistic regression			
Duration of diabetes (years)	1.07	1.01 - 1.13	0.014*
Mean HbA1c (NGSP %)	1.23	1.06 - 1.42	0.006*
Type 1 diabetes	0.65	0.15 - 2.86	0.57
Baseline grade of DR	1.39	1.14 - 1.70	0.001*
Scatter laser treatment†	3.08	1.26 - 7.55	0.014*
Mean sBP (mmHg)	1.02	1.00 - 1.03	0.011*
log[Mean urine ACR] (mg/mmol)	1.43	1.16 - 1.76	0.001*
Mean haemoglobin (g/dl)	0.93	0.74 - 1.16	0.51
HIV positive	0.44	0.10 - 1.94	0.28
Baseline LDL cholesterol (mmol/L)	0.99	0.65 - 1.49	0.95
Baseline HDL cholesterol (mmol/L)	1.81	0.61 - 5.42	0.29
Baseline triglycerides (mmol/L)	1.22	0.96 - 1.55	0.099
Sex (male)	0.68	0.30 - 1.55	0.36
Age (years)	1.02	0.99 - 1.05	0.13
Multivariate logistic regression			
Mean HbA1c (NGSP %)	1.28	1.07 – 1.52	0.006*
Mean sBP (mmHg)	1.01	0.99 – 1.03	0.14
Baseline grade of DR	1.43	0.94 – 2.19	0.097
Baseline triglycerides (mmol/L)	1.27	0.98 – 1.66	0.076
Scatter laser treatment†	0.67	0.11 – 4.11	0.66

† Scatter laser treatment received any time between visit 1 and visit 2

7.4.9 Vision

Visual acuity measurements for 313 subjects seen at baseline and 12 months are shown in Table 7.17. Using WHO definitions [310] the number of subjects at baseline and 12 months with 'normal vision' (equal to or better than 60 letters) was 302 (96.5%, 94.4-98.5) and 299 (95.5%, 93.2-97.8), respectively. The number with 'moderate visual impairment' (50 to 59 letters) was 7 and 7 (2.2%, 0.6-3.9), respectively. The number of 'severely visually impaired or blind' (<50 letters) subjects was 4 (1.2%, 0-2.5) and 6 (1.9%, 0.4-3.4) respectively. At visit 2 the most common primary causes of visual impairment for subjects with corrected visual acuity worse than 80 letters (equivalent to 6/12 Snellen or worse) (n=89) were DR (29.2%) cataract (23.6%), and both DR and cataract (19.1%) (Table 7.18). Therefore in 48.3% of cases DR was the sole or equal contributing cause of visual loss.

Data on visual acuity from both visit 1 and 2 were available for 312 subjects. Between visits 1 and 2, 77 subjects (24.7%) lost 5 or more ETDRS letters of which 16 subjects (5.1%) lost 15 or more letters. 3 subjects (1.0%) progressed to moderate visual impairment (50-59 letters) and 5 (1.6%) became 'severely visually impaired or blind' (<50 letters). The most common primary causes of visual loss for the 77 subjects who lost five or more letters were DR (29%), cataract (18%), and both DR and cataract (8%) (Table 7.19). In 37% of cases DR was the sole or equal contributing cause of visual loss. In univariate analysis loss of 15 or more ETDRS letters was not significantly associated with presence of STDR at visit 2 (OR1.48, 0.52-4.19, p=0.47), age (OR 1.02, 0.98-1.066, p=0.23) or duration of diabetes (OR 1.04, 0.96-1.11, p=0.35).

Table 7.17 Prevalence with 95% CI of corrected ETDRS visual acuities according to better eye in 313 subjects in the MDRS seen at baseline and 12 months. Approximate Snellen acuities in parentheses.

Visual acuity (ETDRS letters)	Visit 1 (baseline)		Visit 2 (12 months)	
	n	% (95% CI)	n	% (95% CI)
≥ 90 (6/5)	86	27.5 (22.5-32.4)	65	20.8 (16.3-25.3)
80 - 89 (6/7.5)	148	47.3 (41.8-52.8)	158	50.5 (44.9-56.0)
70 - 79 (6/12)	60	19.2 (14.8-23.5)	55	17.6 (13.4-21.8)
60 - 69 (6/18)	8	2.6 (0.8-4.3)	21	6.7 (3.9-9.5)
50 - 59 (6/30)	7	2.2 (0.6-3.9)	7	2.2 (0.6-3.9)
40 - 49 (6/75)	2	0.6 (0-1.5)	1	0.3 (0-0.9)
Hand Movements	1	0.3 (0-0.9)	4	1.2 (0-2.5)
Light Perception	1	0.3 (0-0.9)	0	
No light perception	0		1	0.3 (0-0.9)
No data	0		1	0.3 (0-0.9)

Table 7.18 Primary causes of visual impairment (VI) in the opinion of the examining clinician at visit 2 for MDRS subjects with corrected visual acuity worse than 80 letters. Subjects classified according to level of visual impairment (n=89). Approximate snellen equivalents: 70-79 letters = 6/12; 60-69 = 6/18; 50-59 = 6/24 'Moderate visual impairment'; <50 letters = 6/36 or worse 'Severely visually impaired or blind'. AMD = age related macular degeneration. ERM = epiretinal membrane. PCO = posterior capsule opacification.

Primary cause of VI	Level of visual impairment (ETDRS letters)				Total
	70-79	60-69	50-59	<50	
n	55	21	7	6	89
DR	15 (27%)	7 (33%)	2	2	26 (29.2%)
DR and cataract	10 (18%)	5 (24%)	1	1	17 (19.1%)
Cataract	16 (29%)	4 (19%)	0	1	21 (23.6%)
AMD	1 (2%)	1 (5%)	0	0	2 (2.2%)
Glaucoma	1 (2%)	0	1	0	2 (2.2%)
Other	12 (22%)*	4 (19%)‡	3†	2‡	21 (23.6%)

*2 PCO; 5 no cause identified; 1 dry eye; 1 cataract and dry eye; 1 ERM; 2 central foveal scarring

‡ 3 no cause identified; 1 PCO and complicated cat surgery

†1 posterior uveitis (possibly syphilitic); 1 ERM; 1 myopic degeneration and cataract

‡ 1 inherited retinopathy; 1 subject sphenoid meningioma

Table 7.19 Primary causes of visual loss between MDRS visits 1 and 2 in the opinion of the examining clinician for subjects with loss of 5 or more letters. Subjects classified according to number of letters lost and level of visual impairment (n=77). ‘Moderate visual impairment’: 50-59 letters (equivalent to 6/24 Snellen). ‘Severely visually impaired or blind’: <50 letters (equivalent to 6/36 or worse).

Primary cause of visual loss	Number of ETDRS letters lost			Level of visual impairment	
	5-14	≥ 15	Total	Progression to Mod. VI*	Progression to Severe VI†
n	61	16	77	3	5
DR	17	5	22 (29%)	1	2
DR and cataract	5	1	6 (8%)		1
Cataract	10	4	14 (18%)		1
AMD	1	0	1 (1%)		
Glaucoma	2	1	3 (4%)	1	
Other	26‡	5‡	31 (40%)	1†	1*

‡ 1 PCO +complicated cat surgery; 1 dry eye; 1 PCO; 1 central foveal scarring; 22 no cause identified

‡ 1 sphenoid meningioma; 1 ERM; 3 no cause identified

† ERM

* sphenoid meningioma

7.5 Discussion

7.5.1 Principal findings

This chapter details progression of grades of retinopathy and visual impairment over 12 months in a treated cohort of people with diabetes from Southern Malawi. The MDRS is a 24 month cohort study; the 12 month data presented in this chapter is a planned interim analysis. In 313 subjects (88% of the original 357 subject cohort) prevalence of any retinopathy did not change. However, prevalence of STDR and PDR increased from 29.1% (24.1-34.1) to 29.4% (24.4-34.5) and from 6.7% (3.9-9.5) to 7.3% (4.4-10.2), respectively. Incidence at 12 months of any DR in those without evidence of retinopathy at baseline was 11.7% (6.6-16.8). The incidence at 12 months of STDR for those with level 10 and level 20 retinopathy at baseline was 0 and 14.3% (5.7-22.9), respectively. The incidence of PDR for those with level 10, level 20, level 30, level 40 and level 50 retinopathy at baseline was 0, 0, 0, 18% and 38%, respectively. Higher glycosylated haemoglobin (HbA1c) was a risk factor for

progression of retinopathy in multivariate analysis. Over the follow-up period 77 subjects (24.7%) lost 5 or more ETDRS letters of which 16 subjects (5.1%) lost 15 or more letters. In 48% of cases DR was the sole or equal contributing cause of visual loss.

7.5.2 Strengths and weaknesses of this work

The MDRS represents the first prospective cohort study of DR from Sub-Saharan Africa. Strengths and weakness of the study are discussed in relation to both 12 and 24 month data in Chapter 8, Section 8.5.3. The MDRS was powered for the primary endpoint: progression of retinopathy by 2 steps on the LDES scale at 24 months. This 12 month analysis is therefore underpowered with respect to this endpoint.

7.5.3 Analysis of bias

The MDRS achieved a high follow-up rate at 12 months: 88% of the original cohort assessed. 15 subjects (4.2%) were confirmed dead and it is likely that some of the 19 (5.3%) untraced subjects also died. Subjects who were not seen at 12 months were older than those subjects who were seen. Higher mean fasting blood sugar, higher serum creatinine and a higher proportion of subjects with raised serum creatinine in the 'not seen' group may suggest a higher baseline prevalence of microvascular complications of diabetes. Lower mean haemoglobin and higher prevalence of anaemia may reflect poor general health and/or nutrition.

7.5.4 Comparison with previous cohort studies of DR

Few cohort studies are available for comparison from the African continent. Chapter 4, Section 4.4.4 details the only published studies and these are summarised in Table 4.4. These studies report incidence and progression of DR at 5 years and more. They are therefore discussed in relation to MDRS 5 year data in Chapter 9, Section 9.5.4. High quality, prospective cohort studies of DR are available from Europe and North America. Comparison between these studies and MDRS data at 12 and 24 months is made in Chapter 8, Section 8.5.6. The MDRS has

demonstrated an association between glycaemic control and DR progression at 12 months. This association has been shown previously in large, high-quality studies in European and North American populations [12,13,97]. Associations of DR progression in the MDRS at 12 and 24 months are discussed in Chapter 8, Section 8.5.6. Similarly comparisons to studies reporting visual loss in persons with diabetes is made in Chapter 8, Section 8.5.8.

7.6 Chapter summary

This chapter provides an estimate of incidence and progression of grades of retinopathy over 12 months in a treated cohort of persons with diabetes in Southern Malawi. Rates of DR progression were high. Higher glycosylated haemoglobin was a risk factor for progression of retinopathy. The MDRS was powered for the primary endpoint: progression of retinopathy by 2 steps on the LDES scale at 24 months. Chapter 8 describes results from the MDRS at 24 months.

Chapter 8. 24 month Follow-up of the Malawi Diabetic Retinopathy Study Cohort

8.1 Aims of the chapter

This chapter details the demographic characteristics, clinical and biochemical parameters, progression of retinopathy grades and visual acuity data for the Malawi Diabetic Retinopathy Study (MDRS) cohort at 24 month follow-up.

8.2 Introduction

The MDRS is a prospective, observational, cohort study of persons attending 2 hospital diabetes clinics over 24 months. The study aims to describe the prevalence, incidence and progression of diabetic retinopathy (DR) in Southern Malawi and to investigate the determinants of DR severity and progression in this population. The MDRS uses a systematically sampled cohort, standardized clinical photography, independent grading by graders in an accredited reading centre and collects data on covariates specific to the population. In this chapter I report data from this cohort, 24 months after the baseline assessment.

8.3 Methods

8.3.1 Setting, subjects, clinical assessment and subject tracing

Study setting, sampling of subjects, clinical assessment and assessment of retinopathy are described in Chapter 5, Methods. Subject tracing and confirmation of subject death are described in Chapter 7, Section 7.3.2 and 7.3.3, respectively. Subjects were systematically sampled from 2 hospital based, primary care diabetes clinics. At 24 months visual acuity, glycaemic control (HbA1c and fasting blood sugar (FBS)), blood pressure, HIV status, urine albumin-creatinine ratio and haemoglobin were assessed. Retinopathy was graded at an accredited reading centre using modified Wisconsin grading of 4-field mydriatic photographs.

8.3.2 Statistical analysis

An *a priori* analysis plan was followed. As described in Chapter 5 Methods, grades of DR were calculated by patient according to the worse or only gradeable eye. Visual acuity data were investigated by patient according to the better eye. The MDRS was powered for the primary endpoint at 2 years: progression of retinopathy by 2 or more steps on the Liverpool Diabetic Eye Study (LDES) scale. Cumulative and annual incidence rates of grades of DR and sight threatening diabetic retinopathy (STDR) were calculated for one year intervals using the life table method (described in Chapter 5, Section 5.9.10).

I constructed a logistic regression model (backwards stepwise with probability of removal of 0.2) to determine the odds ratio (OR) and 95% CIs for 2 step progression on the LDES scale in association with an initial 12 variables: time since diagnosis of diabetes, type of diabetes, baseline grade of DR, mean HbA1c (mean of visits 1, 2 & 3), mean sBP, mean urine albumin creatinine ratio (ACR), mean haemoglobin, high density lipoprotein (HDL) cholesterol (baseline measurement), triglycerides (baseline), HIV status, age, and scatter laser treatment any time between visit 1 and visit 3. Mean HbA1c and mean BP were used in the analysis as opposed to baseline measurements. A sensitivity analysis using baseline measurements was performed. Data were considered significant when $p < 0.05$. All tests were two-sided. All calculations were performed using STATA version 12 (StataCorp, Texas, USA).

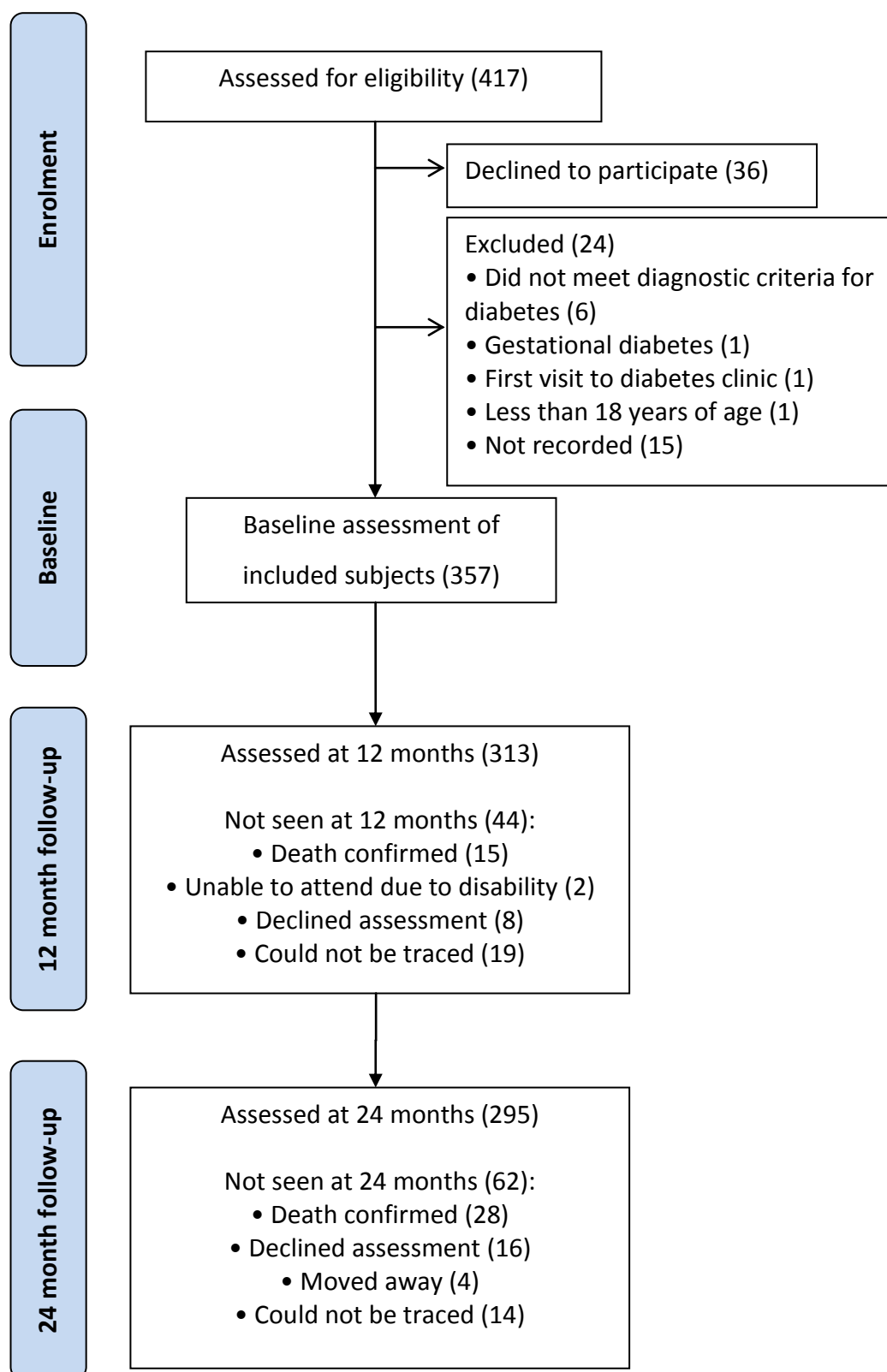
8.4 Results

8.4.1 Participants

A total of 295 subjects were assessed between December 2013 and May 2014 (82.6% of the original 357 subject cohort) (Figure 8.1). The death of 15 subjects was confirmed during the first 12 months of the study (Chapter 7). A further 13 deaths were recorded by 24 months (total 28; 7.8% of original cohort). Therefore total follow-up in the MDRS was 323: 90.5% of the original cohort. 16 subjects were traced but declined assessment. 4 subjects had moved away and were unable to

return for assessment (2 to Lilongwe, 1 to South Africa and 1 to the UK). 14 subjects could not be traced.

Figure 8.1 Flow diagram for subjects in the MDRS: enrolment and follow-up at 12 and 24 months



8.4.2 Subjects confirmed deceased

Baseline characteristics and grades of retinopathy of 28 subjects confirmed deceased by May 2014 are shown in Table 8.1 and 8.2. Three death certificates were available. Causes of death recorded were 'upper gastrointestinal bleeding', 'meningitis' and 'diabetes and ascites'. Four health books were reviewed. Causes of death recorded were 'malaria/hypoglycaemia', 'diabetic ketoacidosis', 'hypoglycaemia/anaemia' and 'heart disease/anaemia'. For 21 subjects the cause of death was assigned based on verbal reports alone: 'diabetic ketoacidosis' 3 subjects, 'renal failure secondary to diabetes' 2, 'brain tumour' 1 (sphenoid meningioma on previous MRI), 'hypoglycaemia' 1, 'anaemia' 1, and 'unknown cause' 13 subjects.

Cumulative incidence of death in the whole MDRS cohort at 12 and 24 months was 4.3% (2.2-6.4 95% CI) and 8.0% (5.1-10.9), respectively (n=357; life table method). Cumulative incidence of death amongst subjects with STDR at baseline at 12 and 24 months was 7.6% (2.6-12.6) and 13.2% (6.8-19.6), respectively (n=106). Cumulative incidence of death amongst subjects with proliferative diabetic retinopathy (PDR) at baseline at 12 and 24 months was 15.4% (1.5-29.3) and 30.8% (13.1-48.6), respectively (n=26). Cumulative incidence of death amongst subjects listed for any laser treatment during the MDRS at 12 and 24 months was 5.8% (0.9-10.7) and 12.8% (5.7-19.9), respectively (n=85). Cumulative incidence of death amongst HIV positive subjects at 12 and 24 months was 10% (1.7-18.3) and 18.1% (7.4-28.8), respectively (n=50). Cumulative incidence of death amongst subjects with moderate visual impairment or worse at baseline (<60 ETDRS letters) at 12 and 24 months was 15% (0-35) and 39% (12-66), respectively (n=13). In univariate analysis death during the MDRS was associated with STDR (OR 2.51; 95% CI 1.15-5.48; p=0.021), PDR (OR 6.47; 2.51-16.7; p=0.0001), HIV (OR 3.72; 1.54-9.00; p=0.003) and moderate visual impairment (OR 8.21; 2.48-27.1; p=0.001).

Table 8.1 Baseline characteristics of 28 subjects from the MDRS cohort confirmed dead by the MDRS team by May 2014. BMI = body mass index. ACR = albumin creatinine ratio.

Characteristic	Level
Female sex	13 (46%)
Age (median, IQR)	56.4 yrs (49.8–64.5)
Type 1 diabetes	4 (14%)
BMI (mean, SD)	23.9 kg/m ² (4.3)
Overweight (BMI>25 kg/m ²)	12 (43%)
Time since diagnosis of diabetes (median, IQR)	5.8 yrs (2.6 – 12.6)
Hypertensive (see text)	15 (54%)
sBP (median, IQR)	128 mmHg (114-158)
dBp (median, IQR)	79 mmHg (72 - 88)
Mean arterial pressure (median, IQR)	96 mmHg (90-109)
HbA1c (NSGP) (mean, SD)	8.7% (3.5)
Fasting blood sugar (mean, SD)	13.7 mg/dL (11.4)
HIV reactive	9 (32%)
Anaemia (WHO definition)	14 (50%) 8M; 6F
Total cholesterol >5.0mmol/L	7 (25%)
Total cholesterol (mmol/L; mean, SD)	3.7 (1.3)
HDL cholesterol (mmol/L; mean, SD)	0.93 (0.39)
LDL cholesterol (mmol/L; mean, SD)	1.98 (0.95)
Triglycerides (mmol/L; mean, SD)	1.45 (0.73)
Urine ACR raised (n; %) (M>2.5/F>3.5 mg/mmol)	19 (68%)
Serum creatinine (mean, SD)	120 µmol/L (125)
Raised serum creatinine (M>110; F>90 µmol/l)	8 (20%)

Table 8.2 Baseline prevalence of grades of retinopathy for 28 subjects from the MDRS cohort confirmed dead by the MDRS team by May 2014. STDR = sight threatening retinopathy.

Grade	n
No retinopathy (level 10)	12 (43%)
Any retinopathy (level 20-71+)	16 (57%)
Level 20 retinopathy	2 (7%)
Level 30 retinopathy	3 (11%)
Level 40 retinopathy	1 (4%)
Level 50 retinopathy	2 (7%)
Proliferative or worse (\geq level 60)	8 (29%)
Ungradeable	0
Sight threatening maculopathy	10 (36%)
STDR	14 (50%)
No data	0

8.4.3 Analysis of bias

In order to determine the degree to which loss to follow-up may have biased results of this cohort study, baseline data from subjects seen at 24 months and those lost to follow-up were compared. Baseline demographic, clinical and biochemical parameters of the 357 subjects in the MDRS cohort categorised by follow-up are shown in Table 8.3. There was no significant difference between subjects seen at 24 months and those not seen at 24 months regarding mean duration of diabetes, HbA1c, sBP, dBP, mean arterial pressure (MAP), BMI, triglycerides and HDL cholesterol. There was no significant difference between the proportions of subjects in each group who were hypertensive, overweight, HIV positive, of female sex, had type 1 diabetes or who had raised cholesterol. Subjects who were not seen at 24 months were older and, at baseline, demonstrated higher mean fasting blood sugar and serum creatinine and lower mean haemoglobin, mean total cholesterol and mean low density lipoprotein (LDL) cholesterol. Amongst subjects not seen at

24 months, at baseline a greater proportion were anaemic, had raised creatinine and had raised urine ACR.

Baseline prevalence of grades of retinopathy for 357 subjects in the MDRS cohort categorised by follow-up are shown in Table 8.4. There was no significant difference between subjects seen at 24 months and not seen at 24 months regarding prevalence of any DR or STDR. However, there was a greater baseline prevalence of PDR amongst those not seen at 24 months. There was no significant trend towards increasing grade of DR in those subjects not seen at 24 months compared to subjects seen at 24 months ($p=0.30$, X^2 test for trend). Table 8.5 shows baseline prevalence of corrected Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuities according to better eye for 357 subjects in the MDRS cohort categorised by follow-up. Those subjects not seen at 24 months demonstrated worse baseline visual acuities ($p=0.0004$, X^2 test for trend).

Table 8.3 Baseline demographic, clinical and biochemical parameters of 357 subjects in the MDRS cohort categorised by follow-up: traced and assessed at 24 months (n=295) or not seen at 24 months (n=62). MAP = mean arterial pressure; FBS = fasting blood sugar.

Characteristic	Subjects seen at 24 months	Subjects not seen at 24mths	p value
n	295	62	NA
Female sex	180 (61.0%)	36 (58.1%)	p=0.671 Fisher's exact
Age (yrs; median, IQR)	53.5 (43.4-60.2)	56.2 (48.7-66.0)	p=0.040* Unp'ed t-test
Type 1 diabetes	29 (9.8%)	6 (9.7%)	p=0.999 Fisher's exact
BMI (kg/m ² ; mean, SD)	26.7 (5.5)	25.2 (5.4)	p=0.051 Unpaired t-test
Overweight (BMI>25 kg/m ²)	168 (56.9%)	31 (50%)	p = 0.328 Fisher's exact
Duration (median, IQR)	4.1 (1.9 – 8.1)	4.5 (2.1 – 8.1)	p=0.583 Wilcoxon r.s.
Hypertensive (see text)	194 (65.8%)	38 (61.3%)	p=0.558 Fisher's exact
sBP (mmHg; median, IQR)	135 (121 - 156)	134 (118 - 159)	p=0.937 Unpaired t-test
dBp (mmHg; median, IQR)	82 (74-91)	80 (74 - 89)	p=0.744 Unpaired t-test
MAP (mmHg; median, IQR)	100 (90 - 111)	97 (90 – 116)	p=0.930 Unpaired t-test
HbA1c (NGSP %)(mean, SD)	7.8 (2.4)	8.0 (3.1)	p=0.573 Unpaired t-test
FBS (mg/dL; mean, SD)	10.0 (5.3)	12.5 (9.9)	p=0.005* Unp'ed t-test
Hb (g/dl) (mean; SD)	14.1 (1.8)	13.0 (2.2)	p=0.0001* Unp'ed t-test
Anaemia (see text)	36 (12.2%)	17 (27.4%)	p=0.005* Fisher's exact
HIV positive	36 (12.2%)	12 (19.4%)	p=0.151 Fisher's exact
Total chol. >5.0mmol/L	88 (30%)	17 (27.4%)	p=0.761 Fisher's exact
Total chol(mmol/L;mean,SD)	4.37 (1.25)	3.98 (1.34)	p=0.028* Unp'ed t-test
LDL chol. (mmol/L;mean,SD)	2.49 (0.93)	2.18 (0.99)	p=0.019* Unp'ed t-test
HDL chol(mmol/L;mean,SD)	0.99 (0.34)	0.96 (0.35)	p=0.530 Unpaired t-test
Triglycerides(mmol/L;mean,SD)	1.57 (1.26)	1.30 (0.65)	p=0.102 Unpaired t-test
Raised uACR (M>2.5;F>3.5mg/mmol)	91 (30.8%)	31 (50%)	p=0.005* Fisher's exact
Serum creat (μmol/L;mean,SD)	61.1 (21.4)	86.9 (89.8)	p=0.0001* Un-p'd t-test
Raised creat (M>110;F>90μmol/l)	12 (4.1%)	8 (12.9%)	p=0.012* Fisher's exact

Table 8.4 Baseline prevalence of grades of retinopathy of 357 subjects in the MDRS cohort categorised by follow-up: traced and assessed at 24 months (n=295) or not seen at 24 months (n=44). ST = sight threatening. STDR = sight threatening diabetic retinopathy.

Grade (n; %; 95% CI)	Subjects seen at 24 months	Subjects not seen at 24 months	p value (Fisher's exact)
n	295	62	
No DR (level 10)	140 (47.5; 41.8-53.2)	37 (59.7; 47.5-71.9)	p = 0.09
Any DR (level 20-71+)	154 (52.2; 46.5-57.9)	25 (40.3; 28.1-52.5)	p = 0.09
Level 20 retinopathy	86 (29.2; 24.0-34.4)	8 (12.9; 4.6-21.2)	
Level 30 retinopathy	22 (7.5; 4.5-10.5)	3 (4.8; 0-10.1)	
Level 40 retinopathy	24 (8.1; 5.0-11.2)	2 (3.2; 0-7.6)	
Level 50 retinopathy	6 (2.0; 0.4-3.6)	2 (3.2; 0-7.6)	
Proliferative(≥level 60)	16 (5.4; 2.8-8.0)	10 (16.1; 7.0-25.3)	p = 0.007*
Ungradable	1 (0.3; 0-0.9)	0	
ST maculopathy	81 (27.5; 22.4-32.6)	12 (19.4; 9.6-29.2)	
STDR	88 (29.8; 24.6-35.0)	17 (27.0; 16.0-38.1)	p = 0.761
No data	0	0	

Table 8.5 Baseline prevalence of corrected visual acuities according to better eye (ETDRS letters) for 357 subjects in the MDRS cohort categorised by follow-up: traced and assessed at 24 months (n=295) or not seen at 24 months (n=62). Approximate Snellen acuities in parentheses.

Visual acuity	295 subjects seen at 24 months		62 subjects not seen at 24 months	
	n	% (95% CI)	n	% (95% CI)
≥ 90 (6/5)	82	27.8 (22.7-32.9)	6	9.7 (2.3-17.1)
80 - 89 (6/7.5)	142	48.1 (42.4-53.8)	29	46.8 (34.4-59.2)
70 - 79 (6/12)	54	18.3 (13.9-22.7)	17	27.4 (16.3-38.6)
60 - 69 (6/18)	9	3.1 (1.1-5.1)	4	6.5 (0.4-12.6)
50 - 59 (6/30)	4	1.4 (0.1-2.7)	4	6.5 (0.4-12.6)
40 - 49 (6/75)	2	0.7 (0-1.7)	1	1.6 (0-4.7)
Hand Movements	1	0.3 (0-0.9)	0	
Light Perception	1	0.3 (0-0.9)	0	
No light perception	0		0	
No data	0		1	1.6 (0-4.7)

8.4.4 Demographics and clinical and biochemical measurements of subjects seen at 24 months

For 295 subjects seen at visit 3 median time to follow-up was 1.9 years (range 1.7-2.3). Amongst these 295 subjects at baseline 36 (12.2%) were HIV positive. By 12 months 2 more subjects were reactive. No further subjects were reactive by visit 3 (24 months). The status of 13 subjects (3.6%) was not known as they declined testing throughout the study. No trend toward worsening renal function was identified: Table 8.6 shows urine ACR measurements at visit 1 and 3.

Table 8.6 Urine albumin creatinine ratio (ACR) measurements for 295 subjects in the MDRS seen at visit 1 (baseline) and visit 3 (24 months).

Characteristic	Visit 1 (baseline)	Visit 3 (24 months)
Urine ACR (mg/mmol; mean; SD)	10.4 (42.5)	8.5 (39.7)
Urine ACR raised (n; %) (M>2.5;F>3.5 mg/mmol)	92 (31.2%) (39 men; 53 women)	77 (26.1%) (32 men; 45 women)
Urine ACR > 30 mg/mmol (n; %)	18 (6.1%) (5 men; 13 women)	12 (4.1%) (1 man; 11 women)

8.4.5 Prevalence of grades of retinopathy at 24 months

Prevalence of grades of retinopathy for 295 subjects seen at visit 3 are shown in Table 8.7.

Table 8.7 Prevalence with 95% CI of retinopathy grades according to worse eye in 295 subjects in the MDRS seen at visit 3. ST = sight threatening.

Grade (n; %; 95% CI)	Visit 1 (baseline)	Visit 2 (12 months)	Visit 3 (24 months)
No retinopathy (level 10)	140 (47.5; 41.8-53.2)	141 (47.8; 42.1-53.5)	108 (36.6; 31.1-42.1)
Any DR (20-71+)	154 (52.2; 46.5-57.9)	143 (48.5; 42.8-54.2)	185 (62.7; 57.2-68.2)
Level 20 retinopathy	86 (29.2; 24.0-34.4)	67 (22.7; 17.9-27.5)	100 (33.9; 28.5-39.3)
Level 30 retinopathy	22 (7.5; 4.5-10.5)	34 (11.5; 7.9-15.1)	31 (10.5; 7.0-14.0)
Level 40 retinopathy	24 (8.1; 5.0-11.2)	20 (6.8; 3.9-9.7)	27 (9.2; 5.9-12.5)
Level 50 retinopathy	6 (2.0; 0.4-3.6)	4 (1.4; 0-2.7)	5 (1.7; 0.2-3.2)
Proliferative (≥ 60+)	16 (5.4; 2.8-8.0)	18 (6.1; 3.4-8.8)	22 (7.5; 4.5-10.5)
Ungradeable	1 (0.3; 0-0.9)	2 (0.7; 0-1.7)	2 (0.7; 0-1.7)
ST maculopathy	81 (27.5; 22.4-32.6)	78 (25.8; 20.8-30.8)	90 (30.5; 25.3-35.8)
STDR	88 (29.8; 24.6-35.0)	85 (28.8; 23.6-34.0)	99 (33.6; 28.2-39.0)
No data	0	9 (3.1; 1.1-5.1)	0

8.4.6 Laser treatment

Table 8.8 details the number of subjects who were listed for, started and completed a course of laser treatment during the course of the MDRS (December 2011 until May 2014). Some subjects required multiple treatments of scatter laser, macular laser or both.

Table 8.8 Number of subjects listed for, started and completed a course of laser treatment during the course of the MDRS (December 2011 until May 2014) classified by grade of DR at the start of the study.

Baseline level of retinopathy	n	Laser photocoagulation (listed/started/completed course)		
		Scatter and macular	Scatter alone	Macular alone
Level 10	177	0	0	1/0/0
Level 20	94	0/0/0	1/1/0	12/11/11
Level 30	25	4/4/3	4/4/4	7/7/7
Level 40	26	17/16/12	3/3/3	2/2/2
Level 50	8	6/6/5	2/2/2	0
Level 60	16	13/13/13	3/2/2	0
Level 70+	10	8/8/7	2/2/2	0
90	1	0	0	0
Total	357	48/47/40	15/14/13	22/20/20

8.4.7 Progression of grades of retinopathy

Of the original 357 subject cohort 322 were seen for at least one further study visit and are included in the progression analysis below. Baseline demographics and clinical and biochemical measurements for these subjects are shown in Table 8.9. Subjects with higher levels of baseline retinopathy were older and had longer duration of diabetes, higher sBP, higher HbA1c and lower haemoglobin. A greater proportion of subjects with high DR grades had raised cholesterol, raised urine ACR, were of female sex and were HIV negative.

Table 8.9 Baseline demographics and clinical and biochemical measurements of 322 subjects in the MDRS who were seen for at least two study visits. Duration = time since diagnosis of diabetes.

	Baseline grade of retinopathy						p value
	Level 10	Level 20	Level 30	Level 40	Level 50	Level 60+	
n†	157	89	22	24	8	21	
Female sex	82 (52%)	59 (66%)	13 (59%)	18 (75%)	7 (88%)	15 (71%)	0.006* X ² for trend
Age (yrs; med,IQR)	53.7 (40.3-59.6)	52.2 (41.0-59.8)	55.3 (45.2-61.1)	55.5 (48.1-60.3)	55.0 (49.4-64.2)	55.3 (50.2-65.5)	0.38 Kruskal-Wallis
Duration (yrs; med,IQR)	3.0 (1.4-5.1)	4.8 (1.9-9.3)	7.6 (4.3-11.1)	7.9 (4.2-12.1)	4.3 (2.0-7.8)	7.4 (4.3-16.3)	0.0001* Kruskal-Wallis
sBP (mmHg;med,IQR)	130 (117-150)	132 (118-148)	140 (126-170)	141 (130-172)	158 (137-173)	166 (135-180)	0.0002* Kruskal-Wallis
HbA1c (NGSP,%;mean;SD)	7.5 (2.6)	8.0 (2.5)	8.7 (2.3)	8.9 (1.9)	7.4 (1.8)	7.8 (1.9)	0.006* Kruskal-Wallis
Haemoglobin (g/dl; mean; SD)	14.4 (1.8)	14.0 (1.6)	14.2 (1.9)	13.6 (1.4)	13.3 (2.3)	12.6 (2.0)	0.020* Kruskal-Wallis
HIV positive	29 (18.5%)	8 (9%)	0	0	3 (38%)	1 (5%)	0.032* X ² for trend
Total cholesterol >5.0mmol/L	38 (24%)	27 (30%)	5 (23%)	10 (42%)	4 (50%)	12 (57%)	0.0007* X ² for trend
Raised urine ACR‡	42 (27%) (M24;F18)	24 (27%) (M6;F18)	10 (45%) (M7;F3)	10 (42%) (M3;F9)	3 (38%) (M1;F2)	15 (71%) (M4;F11)	0.0001* X ² for trend

† 1 subject ungradeable at baseline. ‡Raised urine ACR: Male>2.5 mg/mmol; F>3.5 mg/mmol

Table 8.10 shows cumulative yearly incidence for each year of follow-up for development of any DR, level 30, level 40 DR, sight threatening maculopathy and STDR and progression by 2 and 3 steps on the LDES scale for subjects with no retinopathy (level 10) at baseline. Cumulative yearly incidence of progression to higher grades of DR, development of sight threatening maculopathy and STDR and 2 and 3 step progression for persons with level 20, level 30, and level 40 at baseline are shown in Tables 8.11 through 8.13, respectively. Incidence of STDR ($p=0.0001$), ST maculopathy ($p=0.0001$) and PDR ($p=0.0001$) increased with severity of baseline retinopathy (χ^2 test for trend).

Of 16 subjects with level 60 DR at baseline, at 12 months the following grades of DR were recorded: 2 subjects level 20; 1 level 40; 2 level 50; 6 level 60; 2 level 70; 2 subjects died and 1 was lost to follow-up. At 24 months the following grades of DR were recorded: 1 subject level 20; 1 level 30; 3 level 40; 1 level 50; 4 level 60; a further 1 subject was lost to follow-up and 2 more had died. Of 10 subjects with level 70 or 71 DR at baseline, at 12 months the following grades of DR were recorded: 1 subject level 60; 2 level 70; 5 level 71; 2 subjects had died. At 24 months the following grades of DR were recorded: 1 subject level 40; 3 level 71; 2 level 72; 2 more subjects had died.

Two (or more) step progression (from baseline) was observed either at visit 2 or visit 3 in 69 subjects (21.4%; 95% CI 16.9-25.9); three (or more) step progression in 30 subjects (9.3%; 6.1-12.5). Of 225 subjects without STDR at baseline 23 (10.2%; 6.3-14.2) developed the condition during the study. Of 233 subjects without ST maculopathy at baseline (and whose maculopathy was gradeable) 25 (10.7%; 6.8-14.7) developed the condition during the study. Of 279 subjects not listed for scatter laser at baseline and seen for at least 1 further study visit, 15 (5.4%; 2.8-8.1) developed retinopathy requiring scatter laser by visit 3. Of 278 subjects not listed for macular laser at baseline and seen for at least 1 further study visit, 22 (7.9%; 4.7-11.1) developed maculopathy requiring macular laser by visit 3. Figure 8.2 shows cumulative incidence at 2 years of STDR and PDR and of 2 step and 3-step progression for subjects with level 10, 20, 30, 40 and 50 retinopathy at baseline.

Table 8.10 Life tables showing cumulative yearly incidence of development of any retinopathy, sight-threatening maculopathy, and sight-threatening diabetic retinopathy and of progression by 2 (or more) and 3 (or more) steps on the LDES scale in the worse eye of subjects with diabetes and **no** retinopathy at baseline.

	Any retinopathy				Level 30				Level 40			
T	N	n	C. Inc.	95% CI	N	n	C. Inc.	95% CI	N	n	C. Inc.	95% CI
1	177	18	10.8	6.1-15.5	177	6	3.6	0.8-6.4	177	0	0	
2	138	40	38.0	30.2-45.8	150	3	5.6	1.9-9.3	156	1	0.7	0-2.0

	ST maculopathy ‡				STDR ‡							
T	N	n	C. Inc.	95% CI	N	n	C. Inc.	95% CI				
1	177	0	0		174	0	0					
2	156	5	3.4	0.5-6.3	154	4	2.7	0.1-5.3				

	2+ Step progression				3+ Step progression							
T	N	n	C. Inc.	95% CI	N	n	C. Inc.	95% CI				
1	177	8	4.8	1.6-8.1	177	2	1.2	0-2.9				
2	148	13	13.7	8.2-19.3	154	1	1.9	0-4.1				

T = time from recruitment (years); N = number entering time interval; n = new cases diagnosed during year; C. inc. = cumulative incidence (%); CI = confidence interval; ST = sight threatening; STDR = sight threatening diabetic retinopathy. ‡ - those with sight threatening maculopathy/STDR at baseline omitted from analysis

Table 8.11 Life tables showing cumulative yearly incidence of progression to higher grades of retinopathy and development of sight threatening maculopathy and STDR and of progression by 2 (or more) and 3 (or more) steps on the LDES scale for persons with **level 20** retinopathy at baseline.

	Level 30				Level 40				ST maculopathy ‡			
T	N	n	C. Inc.	95%CI	N	n	C Inc	95% CI	N	n	C Inc	95% CI
1	94	12	13.1	6.2-20.0	94	2	2.2	0-5.2	70	7	10.3	3.1-17.5
2	77	13	27.9	18.6-37.2	87	5	7.9	2.3-13.6	59	9	24.3	13.8-34.8

	STDR ‡				2 Step progression				3 Step progression			
T	N	n	C Inc	95% CI	N	n	C Inc	95% CI	N	n	C Inc	95% CI
1	70	9	13.2	5.2-21.3	94	6	6.5	1.5-11.6	94	2	2.2	0-5.2
2	57	9	27.3	16.4-38.2	83	12	20.3	11.9-28.7	87	7	10.2	3.9-16.5

T = time from recruitment (years); N = number entering time interval; n = new cases diagnosed during year; C. inc. = cumulative incidence (%); CI = confidence interval; ST = sight threatening; STDR = sight threatening diabetic retinopathy. ‡ - those with sight threatening maculopathy/STDR at baseline omitted from analysis

Table 8.12 Life tables showing cumulative yearly incidence of progression to higher grades of retinopathy and development of sight threatening maculopathy and STDR and of progression by 2 (or more) and 3 (or more) steps on the LDES scale for persons with **level 30** retinopathy at baseline.

	Level 40				Level 50				Level 60 +			
T	N	n	C. Inc.	95% CI	N	n	C Inc	95% CI	N	n	C. Inc	95% CI
1	25	6	25.5	7.9-43.1	25	0	0		25	0	0	
2	16	4	44.2	23.5-65.0	22	1	4.5	0-13.2	22	2	9.1	0-21.1

	ST maculopathy ‡				STDR ‡							
T	N	n	C. Inc.	95% CI	N	n	C Inc	95% CI				
1	6	0	0		6	0	0					
2	4	1	25.0	0-67.4	4	1	25.0	0-67.4				

	2 Step progression				3 Step progression							
T	N	n	C Inc	95% CI	N	n	C Inc	95% CI				
1	25	5	21.3	4.8-37.9	25	1	4.3	0-12.5				
2	17	3	35.2	15.2-55.2	21	6	31.6	12.2-51.0				

T = time from recruitment (years); N = number entering time interval; n = new cases diagnosed during year; C. inc. = cumulative incidence (%); CI = confidence interval; ST = sight threatening; STDR = sight threatening diabetic retinopathy. ‡ - those with sight threatening maculopathy/STDR at baseline omitted from analysis

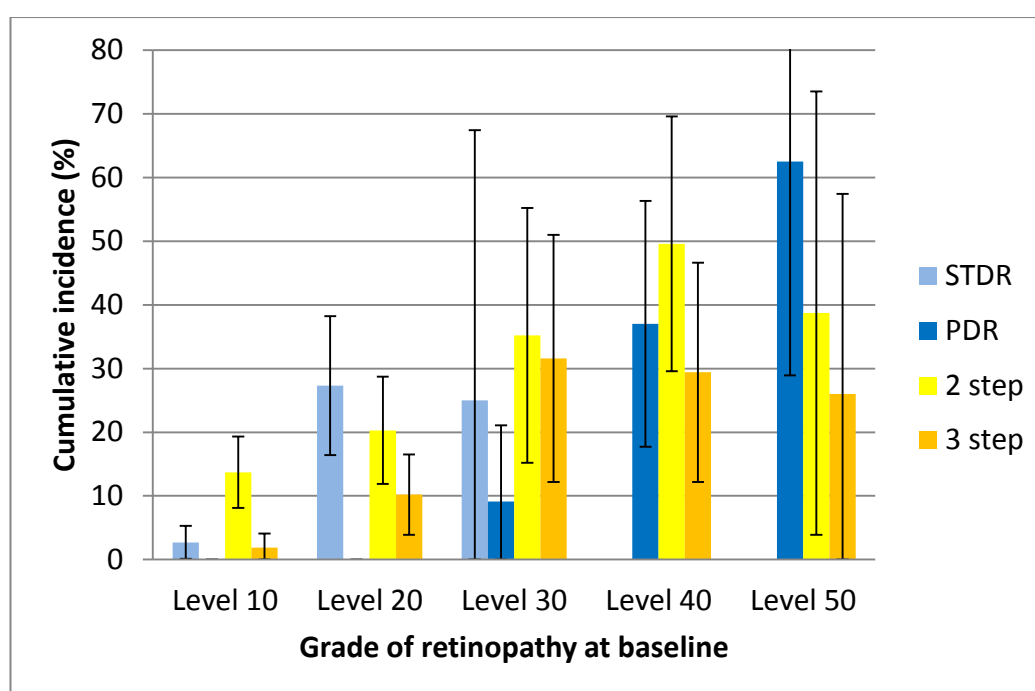
Table 8.13 Life tables showing cumulative yearly incidence of progression to higher grades of retinopathy and development of sight threatening maculopathy and of progression by 2 (or more) and 3 (or more) steps on the LDES scale for persons with **level 40** retinopathy at baseline.

	Level 50				Level 60 +				ST maculopathy ‡			
T	N	n	C. Inc.	95% CI	N	n	C Inc	95% CI	N	n	C Inc	95% CI
1	26	1	4.0	0-11.7	26	4	16	2-30	5	3	60	
2	23	2	13.1	0-27.2	20	5	37	18-56	2	1	80	

	2 Step progression				3 Step progression							
T	N	n	C. Inc.	95% CI	N	n	C Inc	95% CI				
1	26	4	16.0	1.6-30.4	26	4	16.0	1.6-30.4				
2	20	8	49.6	29.6-69.6	20	2	24.4	7.2-41.6				

T = time from recruitment (years); N = number entering time interval; n = new cases diagnosed during year; C. inc. = cumulative incidence (%); CI = confidence interval; ST = sight threatening; STDR = sight threatening diabetic retinopathy. ‡ - those with sight threatening maculopathy at baseline omitted from analysis

Figure 8.2 Cumulative incidence at 2 years of sight threatening diabetic retinopathy (STDR) and proliferative diabetic retinopathy (PDR; level 60+) and of 2 (or more) step and 3 (or more) step progression on the LDES scale for subjects in the MDRS with level 10 (n=177), level 20 (n=94), level 30 (n=25), level 40 (n=26) and level 50 (n=8) retinopathy at baseline. Error bars indicate 95% CI. Classes STDR, PDR, 2 step progression and 3 step progression are not exclusive i.e. a single subject can develop STDR and progress by 2 steps on the LDES scale.



8.4.8 Associations of progression of retinopathy

For 293 subjects seen at visit 3 higher mean HbA1c and higher baseline grade of retinopathy were risk factors for 2 step progression on the LDES scale in multivariate analysis (Table 8.14). HIV infection was negatively associated with progression of DR. Descriptive analysis showed that urine ACR did not demonstrate a linear association with probability of 2 step progression; a logarithmic transformation (base 10) was more suitable for the model.

Table 8.14 Risk factors for association of progression of diabetic retinopathy by 2 or more steps on the LDES scale at 24 months in the MDRS (n=293) (univariate and multivariate logistic regression)

	OR	95% CI	p value
Univariate logistic regression			
Duration of diabetes (years)	1.05	1.00 - 1.09	0.042*
Type 1 diabetes	2.09	0.89 - 4.89	0.090
Baseline grade of DR	1.48	1.23 - 1.76	0.001*
Scatter laser treatment†	3.75	1.90 - 7.40	0.001*
Mean HbA1c (NGSP %)	1.28	1.13 - 1.44	0.001*
Mean sBP (mmHg)	1.00	0.99 - 1.02	0.684
log[Mean urine ACR] (mg/mmol)	1.28	1.07 - 1.52	0.005*
Mean haemoglobin (g/dl)	0.92	0.76 - 1.12	0.432
HIV positive	0.20	0.05 - 0.85	0.029*
Baseline LDL cholesterol (mmol/L)	0.99	0.72 - 1.34	0.925
Baseline HDL cholesterol (mmol/L)	2.24	0.98 - 5.14	0.057
Baseline triglycerides (mmol/L)	0.78	0.57 - 1.06	0.113
Sex (male)	1.04	0.58 - 1.87	0.896
Age (years)	0.99	0.97 - 1.01	0.497
Multivariate logistic regression			
Mean HbA1c (NGSP %)	1.27	1.12 - 1.45	0.001*
Baseline grade of DR	1.39	1.02 - 1.91	0.040*
HIV positive	0.16	0.03 - 0.78	0.023*
Type 1 diabetes	2.27	0.87 - 5.89	0.094
Scatter laser treatment†	1.45	0.43 - 4.88	0.546

† Scatter laser treatment received any time between visit 1 and visit 3

8.4.9 HIV positive subjects

In the MDRS 50 subjects (14.0%) were HIV positive (48 at baseline and 2 new diagnoses during the study). Of these 50 subjects 43 (86%) were seen at visit 2 and 38 (76%) were seen at visit 3. 5 subjects died by 12 months and a further 4 died by 24 months (94% follow-up at 24 months). Characteristics of MDRS subjects classified by HIV status are shown in Table 8.15. HIV positive subjects were younger and demonstrated lower BMI, shorter duration of diabetes, lower sBP and dBP, lower LDL and HDL, higher triglycerides and higher serum creatinine than HIV negative subjects. A higher proportion of HIV positive subjects had raised uACR.

34 subjects (68%) were prescribed antiretroviral therapy (ART) at baseline. One further subject commenced ART during the study. Of 41 HIV positive subjects who underwent annual CD₄ testing 32 (78%), 25 (61%) and 14 (34%) subjects had a CD₄ count below 500, 350 and 200 cells/mm³ at any visit, respectively. The mean CD₄ count was 389 cells/mm³ (SD 242). At 24 months 2 step (or greater) progression was observed in 2/38 HIV positive subjects (5.3%) compared to 55/251 (21.9%) HIV negative subjects ($p < 0.015$ Fisher's exact).

Both subjects who progressed were female. The first subject was diagnosed at baseline visit (WHO clinical stage 2). By visit 2 she had commenced ART. CD₄ counts were: 1106 visit 1; declined blood tests visit 2; 1188 visit 3. Haemoglobin was above 12g/dl at each study visit. Retinopathy grading at baseline and visit 3 were Level 20 DR, Level 0 maculopathy and Level 30 DR, Level 4 maculopathy, respectively. The second subject was taking ART at enrolment. CD₄ counts were: 197 visit 1; 258 visit 2; 202 visit 3. The subject was not anaemic at visits 1 and 2 but haemoglobin dropped to 11.8g/dl at visit 3. Retinopathy grading at baseline and visit 3 were Level 50 DR, Level 4 maculopathy and Level 60 DR, Level 4 maculopathy, respectively. The low numbers of subjects demonstrating progression of retinopathy precludes further analysis of the effects of ART and immune function (as measured by CD₄ count) on progression.

Table 8.15 Demographic clinical and biochemical characteristics of 344 MDRS subjects with known HIV status. Subjects classified by HIV status.

Characteristic	HIV positive	HIV negative	p value
n	50	294	NA
Female sex	24 (48%)	184 (63%)	p=0.061 Fisher's exact
Age (yrs; median, IQR)	48.2 (41.2-56.0)	53.3 (44.0-61.4)	p=0.015* Unp'ed t-test
Type 1 diabetes	5 (10%)	29 (9.9%)	p=0.99 Fisher's exact
BMI (kg/m ² ; mean, SD) ‡	24.7 (5.0)	26.7 (5.6)	p=0.018* Unp'ed t-test
BMI >25kg/m ² ‡	22 (44%)	173 (59%)	p=0.064 Fisher's exact
Duration (median, IQR) ‡	2.8 (0.8-5.3)	4.4 (2.2-8.3)	p=0.002* Wilcoxon r.s.
Mean sBP(mmHg;med,IQR)	121 (112-140)	137 (122-151)	p=0.003* Unp'ed t-test
Mean dBP(mmHg;med,IQR)	77 (70-83)	83 (74-88)	p=0.019* Unp'ed t-test
Mean HbA1c (NGSP%;mean,SD)	8.0 (2.9)	7.8 (2.4)	p=0.60 Unpaired t-test
Mean Hb (g/dl; mean; SD)	13.5 (2.0)	13.7 (1.6)	p=0.43 Unpaired t-test
Anaemia (see text)‡	11 (22%)	40 (13.6%)	p=0.13 Fisher's exact
Total chol. >5.0mmol/L‡	13 (26%)	91 (31%)	p=0.62 Fisher's exact
Total chol‡ (mmol/L;mean,SD)	4.02 (1.43)	4.35 (1.22)	p=0.086 Unpaired t-test
LDL chol.‡ (mmol/L; mean, SD)	2.06 (0.91)	2.50 (0.94)	p=0.002* Unp'ed t-test
HDL chol.‡ (mmol/L;mean,SD)	0.90 (0.33)	1.00 (0.33)	p=0.048* Unp'ed t-test
Triglycerides‡(mmol/L;mean,SD)	2.18 (2.15)	1.43 (0.91)	p=0.0001* Unp'd t-test
Raised uACR‡ (M>2.5;F>3.5mg/mmol)	25 (50%)	93 (31.6%)	p=0.015* Fisher's exact
Serum creatinine‡ (μmol/L;mean,SD)	82.8 (91.7)	63.1 (28.4)	p=0.003* Un-p'ed t-test
Raised creatinine (M>110;F>90μmol/l)	7 (14%)	19 (6.5%)	p=0.079 Fisher's exact

‡ Measurement at baseline

8.4.10 Vision

Visual acuity measurements for 295 subjects seen at baseline and 24 months are shown in Table 8.16. According to WHO definitions [19] the number of subjects at baseline and 24 months with 'normal vision' (equal to or better than 60 letters) was 287 (97.3%, 95.5-99.2) and 289 (98.0%, 96.4-99.6), respectively. The number with 'moderate visual impairment' (50 to 59 letters) was 4 (1.4, 0.1-2.7) and 5 (1.7%, 0.2-3.2), respectively. The number of 'severely visually impaired or blind' (<50 letters) subjects was 4 (1.4, 0.1-2.7) and 1 (0.3%, 0-0.9), respectively. At visit 3 the most common primary causes of visual impairment for subjects with corrected visual acuity worse than 80 letters (equivalent to 6/12 Snellen or worse) (n=118) were DR (38.1%), cataract (22.9%), and both DR and cataract (12.7%)(Table 8.17). Therefore in 50.8% of cases DR was the sole or equal contributing cause of visual loss.

Table 8.16 Prevalence with 95% CI of corrected ETDRS visual acuities according to better eye in 295 subjects in the MDRS seen at baseline and 24 months. Approximate Snellen acuities in parentheses.

Visual acuity (ETDRS letters)	Visit 1 (baseline)		Visit 3 (24 months)	
	n	% (95% CI)	n	% (95% CI)
≥ 90 (6/5)	82	27.8 (22.7-32.9)	34	11.5 (7.9-15.1)
80 - 89 (6/7.5)	142	48.1 (42.4-53.8)	143	48.5 (42.8-54.2)
70 - 79 (6/12)	54	18.3 (13.9-22.7)	78	26.4 (21.4-31.4)
60 - 69 (6/18)	9	3.1 (1.1-5.1)	34	11.5 (7.9-15.1)
50 - 59 (6/30)	4	1.4 (0.1-2.7)	5	1.7 (0.2-3.2)
40 - 49 (6/75)	2	0.7 (0-1.7)	0	
Hand Movements	1	0.3 (0-0.9)	0	
Light Perception	1	0.3 (0-0.9)	0	
No light perception	0		1	0.3 (0-0.9)
No data	0		0	

Table 8.17 Primary causes of visual impairment (VI) in the opinion of the examining clinician at visit 3 for MDRS subjects with corrected visual acuity worse than 80 letters. Subjects classified according to level of visual impairment (n=118). Approximate Snellen equivalents: 70-79 letters = 6/12; 60-69 = 6/18; 50-59 = 6/24 'Moderate visual impairment'; <50 letters = 6/36 or worse 'Severely visually impaired or blind'. AMD = age related macular degeneration; CRVO = central retinal vein occlusion; ERM = epiretinal membrane; PCO = posterior capsule opacification.

Primary cause of VI	Level of visual impairment (ETDRS letters)				Total
	70-79	60-69	50-59	<50	
n	78	34	5	1	118
DR	29	14	2	0	45 (38.1%)
DR and cataract	11	3	1	0	15 (12.7%)
Cataract	18	9	0	0	27 (22.9%)
AMD	3	0	0	0	3 (2.5%)
Glaucoma	0	1	0	0	1 (0.8%)
Other	17*	7‡	2†	1£	27 (22.9%)

* 13 no cause identified; 1 Optic neuropathy; 1 cataract and CRVO; 1 dry eye; 1 ERM

‡ 4 no cause identified; 1 ERM; 1 PCO and complicated cat surgery; 1 central foveal scarring

† 1 posterior uveitis (possibly syphilitic); 1 myopic degeneration and cataract

£ 1 inherited retinopathy

Of 295 subjects seen at baseline and 24 months 127 (43.0%) lost 5 or more ETDRS letters over the course of the study of which 17 subjects (5.8%) lost 15 or more letters. The most common primary causes of visual loss for the 127 subjects who lost five or more letters were DR (38.6%) cataract (16.5%), and both DR and cataract (3.9%)(Table 8.18). Therefore in 42.5% of cases DR was the sole or equal contributing cause of visual loss. In univariate analysis loss of 15 or more ETDRS letters was not significantly associated with presence of STDR at visit 3 (OR1.56, 0.56-4.33, p=0.390), age (OR 0.97, 0.94-1.01, p=0.110), baseline grade of DR (OR 1.28, 0.97-1.69, p=0.084) or duration of diabetes (OR 1.01, 0.94-1.09, p=0.725).

At baseline 8 subjects (2.2%; 95% CI 0.7 - 3.8) had 'moderate visual impairment' (50 to 59 letters; equivalent to 6/24 Snellen), and 5 subjects (1.4%; 95% CI 0.2-2.6) were 'severely visually impaired or blind' (<50 letters; equivalent to 6/36 or worse). By 12 months an additional 3 subjects progressed to moderate visual impairment (having had normal vision at baseline) and 5 became 'severely visually impaired or blind' (3 had normal vision and 2 had moderate visual impairment at baseline). Between visit 2 and visit 3 no further subjects developed moderate or severe visual impairment. The cumulative incidence at 24 months of developing 'moderate VI' or 'severe VI or blindness' for subjects without these conditions at baseline and with at least 1 follow-up visit was 0.9% (0-2.0) and 1.5% (0.2-2.8), respectively (Life table method; n=322).

Table 8.18 Primary causes of visual loss in the opinion of the examining clinician for 127 subjects with loss of 5 or more letters between MDRS visits 1 and 3. Subjects classified according to number of letters lost.

Primary cause of visual loss	Number of letters lost		
	5-14 letters	≥ 15 letters	Total
n	110	17	127
DR	42	7	49 (38.6%)
DR and cataract	5	0	5 (3.9%)
Cataract	20	1	21 (16.5%)
AMD	2	0	2 (1.6%)
Glaucoma	2	0	2 (1.6%)
Other	39 [‡]	9 [‡]	37.8 (%)

[‡] 1 PCO +complicated cat surgery; 1 PCO; 1 cataract and CRVO; 1 dry eye; 1 ERM; 34 no cause identified

[‡] 1 inherited retinopathy; 1 dry eye; 1 ERM; 1 central foveal scarring; 5 no cause identified

8.5 Discussion

8.5.1 Headlines

This study provides critical baseline information on progression of retinopathy and visual impairment in patients attending mixed urban and rural diabetes clinics in Southern Malawi. Over 24 months progression to STDR from 'no retinopathy' and 'background DR' was approximately 4 times and 3 times that reported in recent European studies, respectively. Higher glycosylated haemoglobin and higher baseline grade of DR were risk factors for progression of retinopathy. The negative association of HIV infection with DR progression is a novel finding. Our results highlight the urgent need for provision of services for retinopathy detection and management to avoid a large burden of vision loss.

8.5.2 Principal findings

This chapter details progression of grades of retinopathy and visual impairment over 24 months in a treated cohort of people with diabetes from Southern Malawi. In 295 subjects (83% of the original 357 subject cohort) prevalence of any retinopathy, STDR and PDR increased from 52.2% (95% CI 46.5-57.9) to 62.7% (57.2-68.2), 29.8% (24.6-35.0) to 33.6% (28.2-39.0) and from 5.4 (2.8-8.0) to 7.5 (4.5-10.5), respectively. Cumulative incidence at 24 months of any DR in those without evidence of retinopathy at baseline was 38.0% (30.2-45.8). The cumulative incidence at 24 months of STDR for those with level 10, level 20 and level 30 retinopathy at baseline was 2.7% (0.1-5.3), 27.3% (16.4-38.2) and 25.0% (0-67.4), respectively. The cumulative incidence at 24 months of PDR for those with level 10, level 20, level 30 and level 40 retinopathy at baseline was 0, 0, 9% and 37%, respectively. Higher glycosylated haemoglobin (HbA1c), higher baseline grade of DR and HIV negative status were risk factors for progression of retinopathy in multivariate analysis. Over the follow-up period 127 subjects (43.0%) lost 5 or more ETDRS letters of which 17 subjects (5.8%) lost 15 or more letters. In 42.5% of cases DR was the sole or equal contributing cause of visual loss. The cumulative incidence

at 24 months of developing 'moderate VI' or 'severe VI or blindness' was 0.9% (0-2.0) and 1.5% (0.2-2.8), respectively.

8.5.3 Strengths and weaknesses of this work

The MDRS represents the first prospective cohort study of DR from Sub-Saharan Africa. Strengths of the work include a robust procedure for systematic random sampling of subjects, external validation of retinopathy grading at an accredited reading centre and a comprehensive assessment of systemic parameters including HbA1c, urine ACR and haemoglobin level. Subject follow-up in this region is extremely challenging. The 90.5% follow-up rate is a considerable achievement. The population studied is differentiated from the majority of published studies by location, ethnic makeup and by a high prevalence of infective disease (malaria and HIV) and anaemia. Unlike the majority of previous cross sectional studies from Africa the MDRS provides data on the number of persons requiring laser treatment (new prevalent cases).

The MDRS was a clinic-based study. Conclusions drawn from this work should be generalised with caution. A large population-based study was beyond the scope (and budget) of this PhD fellowship. Many barriers to clinic attendance exist including transportation costs, competing economic tasks (planting and harvesting staple crops), caring for family members and ignorance regarding health, disease and availability of services. Patients who do not attend clinics may be less likely to be diagnosed with diabetes or to comply with therapy. Conversely those with established complications may be more likely to attend clinics and participate in research studies. As explored in Chapter 6, Section 6.4.4, the MDRS included few subjects with diet controlled diabetes. While some patients travel long distances to attend clinics, rural subjects are likely to be underrepresented in our study and form a selected sub-group of the rural diabetes population. One study from Ethiopia suggested a higher prevalence of DR in urban subjects [329] but there are multiple potential confounders to this finding.

Duration of diabetes is a risk factor for DR progression in all major cohort studies [9,83,84,96]. Annual incidence of progression increases with duration of follow-up. For example, in the LDES, for subjects with no retinopathy at baseline, incidence of STDR was 0.3% (0.1-0.5) in the first year rising to 1.8% (1.2-2.5) in the fifth year [9]. In comparison with other cohort studies of diabetic retinopathy, 24 month follow-up is relatively short. Longer follow-up was not possible within this PhD fellowship. However, the assumptions made in the study sample size calculations have been borne out in practice. I estimated a rate of 2 step progression of 20% over 2 years; 21.4% was recorded. I aimed for a sample size of 300 subjects; 322 were seen for a least 1 further study visit; 295 were seen at 2 years. Therefore despite limitations, I believe the size, quality and novel nature of this study and the degree of confidence around the findings render them informative from both an epidemiological and a policy point of view.

8.5.4 Analysis of bias

The high follow-up achieved in the MDRS notwithstanding it is important to assess to what extent the subjects seen at visit 3 are representative of the whole cohort. 28 subjects (7.8%) were confirmed dead and it is likely that some of the 14 (3.9%) untraced subjects also died. Differences between the 'seen' and 'not seen' groups may be predictors of mortality in this population. Regarding baseline demographic and clinical parameters, subjects not seen at visit 3 were older than those who were traced and assessed. Higher mean fasting blood sugar, higher serum creatinine and a higher proportion of subjects with raised serum creatinine, raised urine ACR and PDR in the group who were not seen at visit 3 suggests a higher baseline prevalence of microvascular complications of diabetes. Lower mean haemoglobin, mean total cholesterol and mean LDL cholesterol and higher prevalence of anaemia in the 'not seen' group may reflect poor general health and/or suboptimal nutrition. The 'not seen' group demonstrated worse visual acuities. Poor vision may be a risk factor for mortality either as a marker of microvascular damage in diabetes or as a marker of age. Alternatively poor vision may directly affect an individual's ability to survive in Southern Malawi.

8.5.5 Comparison with African studies

Few cohort studies are available for comparison from the African continent.

Chapter 4, Section 4.4.4 details the only published studies which are summarised in Table 4.4. These studies report incidence and progression of DR at 5 years and more. They are therefore discussed in relation to MDRS 5 year data in Chapter 9, Section 9.5.4.

8.5.6 Comparison with studies in Europe and North America

High quality prospective cohort studies of DR are available from Europe and North America. Table 8.19 summarises selected studies reporting incidence and progression of DR at 1 and 2 years. The first major, high quality, cohort study of DR, the Wisconsin Epidemiological study of Diabetic Retinopathy (WESDR), was performed in the 1980s. Subjects in this study were not assessed annually. The first incidence and progression data were reported at 4 years [83,84]. The WESDR is therefore discussed in relation to MDRS 5 year data in Chapter 9, Section 9.5.5.

Younis et al reported data from 4770 persons with type 2 diabetes [9] and 501 with type 1 diabetes [96] registered with general practices in one English city who had two or more screening events as part of the prospective Liverpool Diabetic Eye Study (LDES). Cumulative incidence of any DR at 2 years in those with no retinopathy (Level 10) at baseline was 14.2% (9.9-18.5) in type 1 diabetes and 10.9% (9.8-11.8) in type 2 diabetes compared to 38.0% (30.2-45.8) in the MDRS. The 2 year cumulative incidence of STDR for those with level 10 and level 20 retinopathy at baseline was 0.8 (0-1.8) , 8.4 (3.6-13.1) in type 1 diabetes and 0.8 (0.5-1.1) and 11.2 (8.9-13.5) in type 2 diabetes, respectively. The corresponding figures in the MDRS were higher: 2.7 (0.1-5.3) and 27.3 (16.4-38.2), respectively. Unlike the MDRS, longer duration of diabetes was associated with progression to STDR in multivariate analysis.

Data on 20,686 persons with type 2 diabetes seen between 1990 and 2006 as part of the English National Screening Program in the county of Norfolk was collected retrospectively and reported by Jones et al [327]. Among subjects without retinopathy at baseline, cumulative incidence at 2 years of background retinopathy (equivalent to LDES level 20) was 12.2% (11.6-12.8), pre-proliferative retinopathy (equivalent to level 40) 0.8% (0.7-1.0) and sight threatening maculopathy 0.11% (0.06-0.19). Among those with background retinopathy at baseline, after 2 years 6.4% (5.4-7.6) developed pre-proliferative retinopathy and 1.27% (0.85-1.89) developed sight threatening maculopathy. Again corresponding figures in the MDRS are higher.

Thomas et al [326] reported retrospective data from persons with type 2 diabetes attending annual screening in Wales with no retinopathy at first screening event. Cumulative incidence of any DR and referable DR (equivalent to LDES level 40, or exudate or thickening within 1 disc diameter (DD) of the centre of the fovea, or circinate or group of exudates within the macula, or any microaneurysm or haemorrhage within 1DD of the centre of the fovea only if associated with a best VA of $\leq 6/12$. i.e. roughly equivalent to STDR as defined in the present study) at 2 years was 21.7% (21.2-22.0) and 0.5% (0.4-0.5), respectively. The above studies are not population-based; a proportion of subjects with diabetes remain undiagnosed in the community. The majority of persons attending DR screening programmes attend a primary care diabetes service. Studies of DR screening programmes therefore represent a good comparison for the MDRS which sampled subjects attending clinics predominantly for primary diabetes care.

Few cohort studies are available from the Asian continent. Lin et al [330] retrospectively collected data on 63,582 subjects with type 2 diabetes from a database of a universal compulsory National Health Insurance program in Taiwan. Subjects attending 3 or more hospital outpatient appointments associated with diabetes were included. Prevalence of 'STDR' (defined as listing for scatter laser treatment, macular laser treatment or pars plana vitrectomy for PDR) was 2.75% for

women and 2.87% for men. Annual incidence ranged between 0.6-1.1% for women and 1.5-2.2% for men.

In summary, compared to recent European studies 2 year incidence of any retinopathy in the MDRS was higher: 38.0% (30.2-45.8) vs estimates between 3.6% [333] and 21.7% [336]. In the MDRS, 2 year progression to STDR from no DR (level 10) was approximately 3 times that reported in recent European studies of screening programmes: 2.7% (0.1-5.3) vs estimates between 0.5% [336] and 0.8% [9,96,331]. Progression to STDR at 2 years from background DR (level 20) was approximately 2.5 times higher: 27.3 % (16.4-38.2) vs estimates between 6.4 [331] and 11.2 [9]. Differences between figures from the MDRS and recent European work are likely to reflect multiple disparities between populations including ethnicity, access to health services and presence of comorbidities including poorly controlled hypertension and infective disease.

Table 8.19 Summary table of selected studies reporting incidence and progression of DR at 1 and 2 years.

Study		Sweden [332]	Norfolk [331]	LDES [96]		LDES [9]		UK [333]		Wales [336]	MDRS
Dates		1986-1996	1990-2006	1991-1999	1991-99	2000-2008		2005-2009	2012-2014		
Type of study		Hospital clinic-based cohort	Retrospective analysis of screening prog.	Prospective study of DR screening programme		Retrospective analysis of primary care attendees from diagnosis of diabetes		Retrospective analysis of DR screening prog.	Primary care based cohort		
Type of diabetes		Type 1	Type 2	Type 1	Type 2	Type 1	Type 2	Type 2	Type 1 & 2		
n		452	20,686	501	20,570	1,757	63,226	49,763	357		
Cumulative Incidence of any DR (%) †	1 yr	6	1.6	7.8 (4.7–10.9)	5.3 (4.6–6.0)	2.0 (1.4-2.8)	3.8 (3.7-4.0)	12.5 (12.1-12.8)	10.8 (6.1-15.5)		
	2 yrs	15	13.1	14.2 (9.9–18.5)	10.9 (9.8–11.8)	3.6 (2.8-4.6)	6.4 (6.2-6.6)	21.7 (21.2-22.0)	38.0 (30.2-45.8)		
Cumulative Incidence of STDR (%)	1 yr	9	0.1 {L10} [*] 1.1 {L20} [*]	0.3 {L10} 3.6 {L20} 13.5 {L30}	0.3 {L10} 5.0 {L20} 15.0 {L30}			0.2 {L10}	0 {L10} 13.2{L20} 0 {L30}		
	2 yrs	15	0.8 {L10} [*] 6.4 {L20} [*]	0.8 {L10} 8.4 {L20} 33.3 {L30}	0.8 {L10} 11.2 {L20} 27.8 {L30}			0.5 {L10}	2.7 {L10} 27.3 {L20} 25.0 {L30}		
Cumulative Incidence of PDR (%)	1 yr		0.01 {L10} 0.27 {L20}					0 {L10}	0 {L10} 0 {L20} 0 {L30} 16 {L40}		
	2 yrs		0.13 {L10} 1.58 {L20}					0.02 {L10}	0 {L10} 0 {L20} 9.1 {L30} 37{L40}		

{ } Subgroup or baseline LDES retinopathy grade: L10 = level 10, L20 = level 20 etc. †95% confidence interval in parentheses. * Incidence of Level 40 retinopathy only (sight threatening maculopathy data not included). MDRS = Malawi diabetic retinopathy study; LDES = Liverpool diabetic eye study; DR Diabetic retinopathy; STDR = Sight threatening diabetic retinopathy; PDR = Proliferative diabetic retinopathy.

8.5.7 Associations of DR progression

The MDRS has demonstrated an association between glycaemic control and DR progression. This association has been shown previously in large, high-quality studies in European and North American populations [12,13,97]. An association between baseline grade of DR and progression was shown in the MDRS which has also been shown previously [9,83,84,96]. The WESDR also showed an association between baseline grade of DR and visual loss [85] but this is likely to be less relevant in the 2010s since the introduction of anti-VEGF therapy. Consistent associations between DR progression and blood pressure [14,157,315,316] as well blood lipid levels [15] have been shown in European and North American studies. The MDRS showed no evidence for these associations in a Southern Malawian population. However, my analysis does not rule out effects of these variables. In the MDRS mean urine ACR was associated with progression of DR in univariate but not multivariate analysis. Interestingly, in contrast to retinopathy, there was no evidence of an overall decline in renal function over 24 months (Section 8.4.4 above). Subjects with worsening renal function may have been lost to follow-up or died. The contrasting patterns seen with retinopathy and nephropathy may reflect that advanced nephropathy is more immediately fatal than retinopathy.

This study has demonstrated a negative association between DR progression and HIV infection, a novel finding. An important potential confounder of this relationship is early diagnosis of diabetes in HIV positive subjects. Patients attending medical facilities for ART treatment may be more likely to be tested for diabetes than the general population. While the logistic regression analysis presented above controls for known duration of diabetes the duration of diabetes before diagnosis is not known. Both HIV infection and anti-retroviral therapies are associated with a vasculopathy which manifests as increased cardiovascular and cerebrovascular risk [326,327]. Low grade proteinuria is highly prevalent in HIV positive patients taking ART and is more common in persons with concomitant diabetes [334]. A previous cross-sectional study of diabetes complications in Blantyre [1] showed a higher prevalence of proteinuria in subjects with diabetes

and HIV compared to those with diabetes alone. My data does not support an increased risk of all diabetic microvascular complications in HIV.

8.5.8 Vision

To my knowledge no studies from Africa have reported longitudinal visual acuity (VA) data in subjects with diabetes. Specifically the Mauritius DR cohort study [215] reported VA at baseline but not in the follow-up assessment. Incidence of visual impairment (VI) was reported for persons with type 1 diabetes in the WESDR [85]. During the first 4 years of follow-up the annual incidence of VI (defined as best-corrected VA in the better eye of 6/12 or worse) and severe visual impairment (6/60 or worse) was 0.4% and 1.2%, respectively. Hall et al [335] studied blind registrations attributed to DR in Scotland. These authors estimated that the annual incidence of blindness (defined as visual acuity in the better eye below 3/60) in the population with diabetes was 0.04%. In the MDRS the cumulative incidence at 24 months of developing 'moderate VI' (50 to 59 letters; equivalent to 6/24 Snellen) or 'severe VI or blindness' (<50 letters; equivalent to 6/36 or worse) was 0.9% (0-2.0) and 1.5% (0.2-2.8), respectively. While the MDRS was an observational cohort study, subjects could take up a variety of medical interventions which could improve VA most notably laser photocoagulation for DR and cataract surgery. It is likely that a greater degree of visual loss would have been recorded without these interventions.

8.6 Chapter summary

This chapter provides an estimate of incidence and progression of grades of retinopathy over 24 months in a treated cohort of persons with diabetes in Southern Malawi. In this cohort 2 year progression to STDR from 'no DR' and 'background DR' was approximately 4 times and 3 times that reported in recent European studies, respectively. Higher glycosylated haemoglobin and higher baseline grade of DR were risk factors for progression of retinopathy. The negative association of HIV infection with DR progression is a novel finding. When indicated, MDRS subjects had access to laser treatment for DR, a factor likely to have

mitigated DR progression and visual loss in this cohort. Progression of retinopathy and visual loss in an untreated cohort of subjects with diabetes is considered in Chapter 9.

Chapter 9. Progression of diabetic retinopathy at 5 years

9.1 Background to the study of retinopathy progression at 5 years

A cross sectional study of complications of diabetes was conducted at the Queen Elizabeth Central Hospital (QECH) diabetes clinic in 2007 [1]. Of 620 subjects included in the study 281 were examined for retinopathy by an ophthalmologist. The results of this sub-study have been published [2] and are referred to in Chapter 4, Section 4.4.5 and Chapter 5, Section 5.10.1. At this time laser treatment was not available in the public sector in Blantyre. A laser was donated to Lions First Sight Eye Unit in 2010 by the World Diabetes Foundation, however, few persons were treated in 2010 or 2011. Our group were aware that recall of the 2007 subjects would be an extremely valuable opportunity to assess progression of retinopathy and visual loss over 5 years in a population not exposed to laser treatment.

9.2 Chapter aims

In this part of the Malawi Diabetic Retinopathy Study (MDRS) I aimed to trace as many of the 2007 subjects as possible, document grades of diabetic retinopathy (DR) and thereby assess progression of retinopathy over 5 years, report associations of progression with systemic parameters and document visual loss and causes of visual loss in an untreated cohort.

9.3 Methods

9.3.1 Study setting

The diabetes clinic at QECH is described in Chapter 5 'Methods'. In the period 2007 to 2012 the clinic has undergone a number of changes. The number of registered patients has increased from approximately 800 to 2000. A vibrant nurse-led patient education programme supported by the World Diabetes Foundation commenced in 2008. Its aims are improving compliance with diet and medications and educating patients on the complications of diabetes. An electronic records system (Diabetes

and Hypertension System, Baobab Health Trust, Malawi) was installed in early 2010. This runs in parallel to the 'Health passport' system described in Chapter 5.

In 2007 medications regularly available free of charge were glibenclamide and insulin (lente and soluble). Metformin was available from private pharmacies but rarely from the hospital pharmacy. By 2011 metformin was more frequently available free of charge. However, supplies of all drugs remain intermittent. Tests available at the clinic were the same in 2007 as 2012. Glycaemic control is measured by fasting blood sugar on the day of clinic and BP, height and weight are measured by nursing staff. Measurement of lipids, glycosylated haemoglobin and urine test sticks for microalbuminuria are not available routinely.

9.3.2 Subjects

Consecutive patients attending for routine out-patient review between March and June 2007 were invited to participate in the original cross sectional study of complications of diabetes. Of 620 subjects included in the study 281 were examined for retinopathy by an ophthalmologist. Sampling was *ad hoc*: subjects had slit lamp examination if the ophthalmologist was present at the particular clinic at which they were recruited.

9.3.3 Tracing of subjects from the 2007 cohort

The 2007 study was not planned as a cohort study. Subject names and date of birth were recorded and a sticker placed in their 'health passport'. No contact details were recorded. The population of Blantyre is extremely fluid. Migration levels between rural and urban environments and between towns are known to be high. It is common for people who become disabled (e.g. as a result of a stroke) to relocate to be cared for by family members. There exists no mechanism for tracing subjects who fail to attend a clinic appointment. Although no formal study has assessed mortality rate in the QECH diabetes clinic population it is believed to be high. Many persons die at home and there is no formal system for registering deaths. Subject tracing was therefore expected to be extremely difficult.

Tracing of subjects was systematic. All subjects recruited to MDRS main cohort were asked about participation in the 2007 study and their health passports checked for the study sticker. The QECH diabetes clinic electronic patient record system was searched by subject name by the MDRS study team. Identified persons were then contacted by phone. The MDRS study team attended the diabetes clinic weekly between May and November 2012 to approach patients in the clinic waiting room. Finally the President and Vice President of the patient's organisation the Malawi Diabetes Association reviewed the list of subjects from 2007 in order to personally identify subject whereabouts. Written informed consent was obtained from all subjects prior to enrolment.

9.3.4 Confirmation of subject death

Confirmation of subject death was performed in a systematic manner and is described in Chapter 5, Section 5.10.5. The relatives of subjects reported to be deceased were visited at home by the study research nurse between May and November 2012. The nurse was trained to record, on a standard form, brief written narratives from families or other reliable informants. If available the death certificate and the health passport were reviewed and cause of death and/or brief details of last illness recorded. A subject was recorded as dead if confirmed by a relative or 'Traditional authority' (village leader in rural districts), or if a death certificate or marked grave was seen by the study nurse. I assigned a probable cause of death after reading the form.

9.3.5 Clinical assessment

In the 2007 study a self-reported questionnaire was completed with the assistance of a research assistant and with reference to the subject's 'health passport'. Data was collected on demographic details, diet, past medical history and medications. Diabetes with young age at onset and early use of insulin was deemed to be type 1 with all others as type 2. Subjects with type 2 diabetes were sub-classified based on treatment: insulin-requiring with or without oral hypoglycaemic agents, oral

hypoglycaemic agents alone or dietary measures alone. For the purposes of the analysis below the 2007 classification of type 1 and type 2 diabetes was retained i.e. subjects were not reclassified in 2012.

A physical examination by a trained clinician included BP, body mass index (BMI) and neurovascular assessment (abridged four point monofilament examination with a 10 g monofilament [312]. Visual acuity (VA) (corrected with pin-hole) was measured using a Snellen chart. Fasting blood sugar (FBS), glycosylated haemoglobin (HbA1c) and HIV status were tested. A subgroup of subjects were also tested for microalbuminuria and serum creatinine. In 2012 all subjects were assessed in the same manner as subjects of the main MDRS cohort. Clinical assessment is described in Chapter 5 'Methods'. Subjects were classified as hypertensive according to the WHO Steps survey definition [17]: subject either taking antihypertensive medication, or systolic blood pressure (sBP) ≥ 140 mmHg, or diastolic blood pressure (dBp) ≥ 90 mmHg. Thresholds for anaemia were set according to WHO guidelines: 13.0 g/dL for men; 12.0 g/dL for women [307].

9.3.6 Assessment of retinopathy

In 2007 slit lamp biomicroscopy retinopathy grading with 90 and 60 D lenses was performed by one experienced ophthalmologist (Mr. Simon Glover) [2]. Pupils were dilated with 1% tropicamide +/- 10% phenylephrine. In 2012 all subjects were assessed in the same manner as subjects of the main MDRS cohort. Assessment of retinopathy is described in Chapter 5 'Methods'. In both 2007 and 2012 retinopathy and maculopathy were classified by feature specific grading using definitions established in the Liverpool Diabetic Eye Study (LDES)[94] (Chapter 5, Table 5.1). The 2012 grading procedure was more robust: dual grading with arbitration of digital fundus photography of four 45° standard fields [94] performed by accredited graders at a recognised reading centre (considered a reference standard for DR grading). In contrast 2007 grading was clinical (performed at the slit lamp) with no external validation procedure.

9.3.7 Statistical analysis

The 2007 study was not planned as a cohort study. Therefore no relevant power calculation was performed. As in the main cohort study grades of DR were calculated by patient according to the worse or only gradable eye. Visual acuity data were investigated by patient according to the better eye. 95% confidence intervals (CIs) were calculated for proportions. Demographic and clinical differences were assessed at baseline with the unpaired t-test, Wilcoxon rank sum test, Fisher's exact test or χ^2 test for trend. All tests were two-sided and data were considered significant when $p < 0.05$. All Calculations were performed using STATA version 12 (StataCorp, Texas, USA).

I constructed a logistic regression model (backwards stepwise with probability of removal of 0.2) to determine the odds ratio (OR) and 95% CIs for the primary end point (5 years): progression of DR by 2 steps on the Liverpool Diabetic Eye Study (LDES) scale. Where possible the baseline or mean measurement (mean of measurements at 2007 and 2012 visits) was used for each variable in the analysis. For those variables only measured at the 2012 visit this value was used. An initial 11 variables were studied: mean HbA1c, duration of diabetes, baseline grade of DR, type of diabetes, mean sBP, haemoglobin level (2012), urine albumin creatinine ratio (ACR)(2012), triglycerides (2012), HIV status, age and sex. I constructed a second logistic regression model to determine the OR and 95% CIs for the secondary endpoint (5 years): progression to STDR using the same initial explanatory variables.

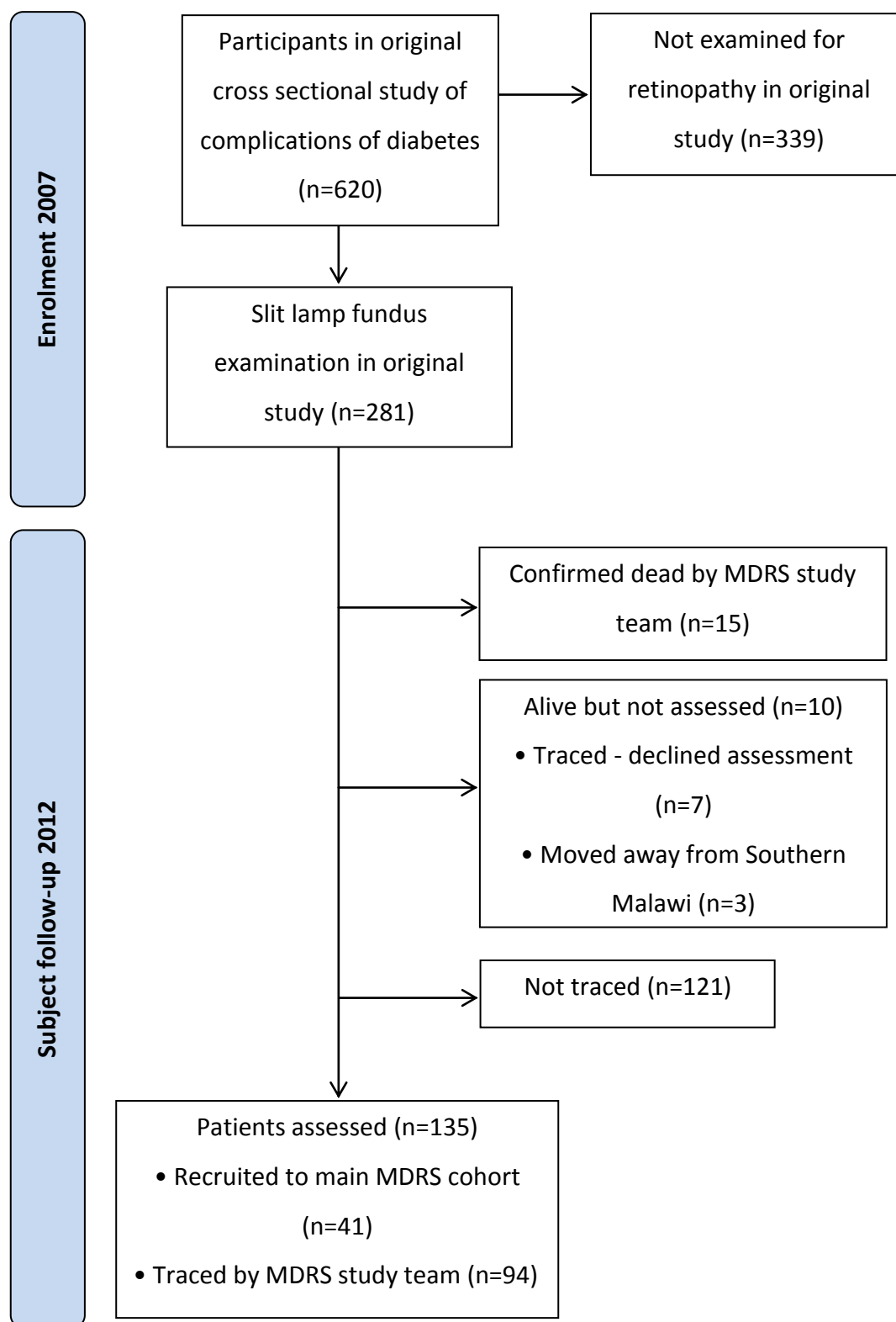
9.4 Results

9.4.1 Participants

A total of 135 subjects were assessed (48.0% of the original 281 subject cohort) (Figure 9.1). Of this group, 41 subjects were recruited to the MDRS cohort study (Chapter 6) between December 2011 and May 2012. Additionally 94 subjects were traced and seen between July 2012 and January 2013. 15 were traced and confirmed as dead. 7 subjects were traced but declined assessment. 3 subjects had

moved away from Southern Malawi and were unable to return for assessment. 121 subjects could not be traced.

Figure 9.1. Flow diagram for tracing of subjects from the 2007 QECH survey of diabetes complications.



9.4.2 Subjects confirmed deceased in 2012

15 subjects from the original 2007 study were confirmed deceased by the MDRS study team in 2012. Baseline characteristics of these subjects are shown in Table 9.1. Baseline grades of retinopathy for these subjects are shown in Table 9.2. No death certificates were available; for 5 subjects the health book was reviewed while for 10 subjects the cause of death was assigned based on verbal reports alone. Cause of death was recorded as: ketoacidosis 1 subject; sepsis 1; renal failure secondary to diabetes 1; hypoglycaemia 2; and unknown cause 10 subjects.

Table 9.1 Baseline characteristics (2007) of 15 subjects with diabetes confirmed dead by the MDRS team in 2012

Characteristic	Level
Female sex	9 (60%)
Age (median, IQR)	60 yrs (56.5 – 61.5)
Type 1 diabetes	0
BMI (mean, SD)	25.4 kg/m ² (4.4)
Overweight (BMI>25 kg/m ²)	9 (60%)
Duration of diabetes (median, IQR)	8.7 yrs (6.3 – 13.7)
Hypertensive (see text)	11 (73%)
sBP (median, IQR)	160 mmHg (120-171)
dBp (median, IQR)	90 mmHg (65-100)
Mean arterial pressure (median, IQR)	113 mmHg (83.3-123.6)
HbA1c (NSGP) (mean, SD)	9.9% (2.3)
Fasting blood sugar (mean, SD)	199.9 mg/dL (96.9)
HIV reactive	1 (6.6%)
Urine dipstick	None 2; Trace 3; 1+ 3; 2+ 3; 3+ 2; 4+ 0
Serum creatinine (mean, SD)	98.6 µmol/L (57.5)
Raised serum creatinine (M>110;F>90 µmol/l)	3 (20%)

Table 9.2 Baseline prevalence of grades of retinopathy (2007) for 15 subjects with diabetes confirmed dead by the MDRS team in 2012.

Grade	n (%) (95% CI)
No retinopathy (level 10)	6 (40%) (15.2 – 64.8)
Any retinopathy (level 20-71+)	9 (60%) (35.2 – 84.8)
Level 20 retinopathy	3 (20%) (0 – 40.2)
Level 30 retinopathy	3 (20%) (0 – 40.2)
Level 40 retinopathy	2 (13%) (0 – 30.0)
Level 50 retinopathy	0
Proliferative or worse (\geq level 60)	1 (7%) (0 – 19.9)
Ungradeable	0
Sight threatening maculopathy	10 (67%) (43.2-90.8)
STDR	10 (67%) (43.2-90.8)
No data	0

9.4.3 Analysis of bias

Baseline demographic, clinical and biochemical characteristics of 281 subjects included in the 2007 study categorised by follow-up are shown in Table 9.3. These comparisons are exploratory and p-values are not corrected for multiple comparisons. There was no significant difference between subjects seen in 2012 and those not seen in 2012 regarding mean BMI, dBP, HbA1c, FBS, serum creatinine or duration of diabetes or concerning grades of proteinuria on urine dipstick. There was no significant difference between the proportions of subjects in each group who were overweight, hypertensive, HIV positive, of female sex or who had type 1 diabetes. There was evidence of a difference between the two groups regarding mean age, sBP and mean arterial pressure (MAP) and concerning the proportion of subjects with raised creatinine.

Baseline prevalence of grades of retinopathy of 281 subjects included in the 2007 study categorised by follow-up are shown in Table 9.4. There was no significant difference between subjects seen in 2012 and those not seen in 2012 regarding

prevalence of any DR and proliferative DR (PDR). There was evidence of a difference between the two groups with regard to STDR. Table 9.5 shows baseline prevalence of corrected Snellen visual acuities according to better eye for 281 subjects included in the 2007 study categorised by follow-up. There was a significant difference between the two groups ($p = 0.0001$, χ^2 test for trend).

Table 9.3 Baseline (2007) demographic, clinical and biochemical parameters of 281 subjects included in the 2007 study categorised by follow-up: traced and assessed in 2012 ($n=135$) or not seen in 2012 ($n=146$). MAP = mean arterial pressure.

Characteristic	Subjects not seen in 2012	Subjects seen in 2012	p value
n	146	135	NA
Female sex	97 (66.4%)	93 (67.4%)	$p = 0.703$ Fisher's exact
Age (yrs; med, IQR)	57.5 (48-65)	52.0 (45-58)	$p=0.0014^*$ Unp'ed t-test
Type 1 diabetes	14 (9.6%)	18 (13.3%)	$p = 0.352$ Fisher's exact
BMI (kg/m^2 ; mean, SD)	28.6 (5.8)	29.4 (5.9)	$p=0.2529$ Unpaired t-test
BMI $>25 \text{ kg/m}^2$	100 (68.5%)	102 (75.6%)	$p = 0.2319$ Fisher's exact
Duration (yrs; med, IQR)	5.7 (1.8 - 9.8)	3.93 (2.2 – 7.8)	$p = 0.234$ Wilcoxon r.s.
Hypertensive (see text)	107 (73.3%)	94 (69.6%)	$p = 0.511$ Fisher's exact
sBP (mmHg; med, IQR)	140 (120-160)	130 (120-150)	$p = 0.015^*$ Unp'red t-test
dBp (mmHg; med, IQR)	88 (70-92)	80 (70-90)	$p = 0.117$ Unpaired t-test
MAP (mmHg; med, IQR)	103.3 (90.0-113.3)	96.7 (89.3 – 108.2)	$p = 0.037^*$ Unp'red t-test
HbA1c (NGSP%;mean,SD)	9.3 (2.2)	9.4 (2.6)	$p = 0.727$ Unpaired t-test
FBS (mg/dL; mean, SD)	194.9 (94.3)	183.8 (88.0)	$p = 0.310$ Unpaired t-test
HIV positive	15 (10.2%)	15 (11.1%)	$p=0.849$ Fisher's exact
Urine dipstick	None 29; trace 42; 1+ 25; 2+ 11; 3+ 6; 4+ 3	None 43; trace 44; 1+ 29; 2+ 6; 3+ 5; 4+ 1	$p = 0.06 \chi^2$ test for trend
Serum creatinine ‡	88.3 (31.7)	83.2 (21.7)	$p = 0.120$ Un-p'red t-test
Raised creatinine†	29 (19.9%)	15 (11.1%)	$p = 0.049^*$ Fisher's exact

†Raised serum creatinine: men $>110 \mu\text{mol/l}$; women $>90 \mu\text{mol/l}$. ‡ $\mu\text{mol/L}$; mean, SD

Table 9.4 Baseline (2007) prevalence of grades of retinopathy of 281 subjects included in the 2007 study categorised by follow-up: traced and assessed in 2012 (n=135) or not seen in 2012 (n=146). ST = sight threatening

Grade (n; %; 95% CI)	Subjects not seen in 2012	Subjects seen in 2012	p value (Fisher's exact)
n	146	135	
No DR (level 10)	94 (64.4; 56.6-72.2)	93 (68.9; 61.1-76.7)	p = 0.450
Any DR (level 20-71+)	48 (32.9; 25.3-40.5)	40 (29.6; 21.9-37.3)	p = 0.607
Level 20	14 (9.6; 4.8-14.4)	23 (17.0; 10.7 – 23.4)	
Level 30	15 (10.3; 5.4-15.2)	9 (6.7; 2.5 – 10.9)	
Level 40	7 (4.8; 1.3-8.3)	5 (3.7; 0.5-6.9)	
Level 50	1 (0.7; 0-2.0)	0	
Proliferative (≥level 60)	11 (7.5; 3.2-11.8)	3 (2.2; 0 – 4.7)	p = 0.054
Ungradable	4 (2.7; 0.1-5.3)	2 (1.5; 0 – 3.5)	
ST maculopathy	28 (19.2; 12.8-25.6)	12 (8.9; 4.1 – 13.7)	
STDR	35 (24.0; 17.1-30.9)	17 (12.6; 7.0-18.2)	p = 0.021*
No data	0	0	

Table 9.5. Baseline (2007) prevalence with 95% CI of corrected Snellen visual acuities according to better eye for 281 subjects included in the 2007 study categorised by follow-up: traced and assessed in 2012 (n=135) or not seen in 2012 (n=146).

Visual acuity	146 subjects not seen in 2012		135 subjects seen in 2012	
	n	% (95% CI)	n	% (95% CI)
6/5	8	5.5 (1.8-9.2)	8	5.9 (1.9-9.9)
6/6	17	11.6 (6.4-16.9)	37	27.4 (19.9-34.9)
6/9	41	28.1 (20.8-35.4)	50	37.0 (28.9-45.2)
6/12	26	17.8 (11.6-24.0)	17	12.6 (7.0-18.2)
6/18	20	13.7 (8.1-19.3)	15	11.1 (5.8-16.4)
6/24	7	4.8 (1.3-8.3)	3	2.2 (0-4.7)
6/36	6	4.1 (0.9-7.3)	0	
6/60	5	3.4 (0.5-6.4)	3	2.2 (0-4.7)
Hand Movements	4	2.7 (0.1-5.4)	0	
Light Perception	4	2.7 (0.1-5.4)	0	
No light perception	2	1.4 (0-3.3)	0	
No data	6	4.1 (0.9-7.3)	2	1.5 (0-3.5)

9.4.4 Demographics and clinical and biochemical measurements of subjects seen in 2012

Demographic, clinical and biochemical characteristics for 135 subjects seen in the 2007 study and subsequently traced and assessed by the MDRS team in 2012 are shown in Table 9.6. 93 subjects (67.4%) were female. Median time to follow up was 5.3 years (range 4.7 - 5.8 years). 18 subjects (13.3%) had type 1 diabetes. Of 117 subjects with type 2 diabetes, at baseline (2007) 22 (18.8%) were prescribed insulin (with or without oral hypoglycaemics), 88 (75.2%) were managed with oral hypoglycaemics and 7 (6.0%) were managed with diet alone. At the follow-up visit (2012) 40 (34.2%) were prescribed insulin, 70 (59.8%) were managed with oral hypoglycaemics and 7 (6.0%) were managed with diet alone. In 2012 97 (71.9%) subjects were HIV non-reactive. 17 (12.6 %) were reactive: 13 taking ART; 3 known HIV+ but not taking ART; and 1 new diagnosis (WHO stage 3). 21 subjects (15.6%) declined testing. 2 subjects seroconverted during the follow-up period. 8 men (19.0 %) and 18 women (19.4 %) were anaemic as defined above. Of the whole cohort 89 subjects were taking antihypertensive medications. Additionally 9 had either sBP ≥ 140 mmHg or dBP ≥ 90 mmHg.

At baseline (2007) the following complications of diabetes were recorded in health passports or reported by subjects: stroke 2 subjects (1.5%), ischaemic heart disease 0. On examination 7 subjects had foot ulcers but none had amputations; 27 (%) had objective sensory neuropathy on abridged four point monofilament examination. At the follow-up visit (2012) the following complications of diabetes were recorded in health passports or reported by subjects: stroke 8 (5.9 %); amputations 3 (2.2 %); erectile dysfunction 21 (50.0 % of men); and ischaemic heart disease 3 (2.2 %). 27 (20.0 %) subjects reported an episode of malaria in the past 12 months; 6 (4.4%) and 3 (2.2%) reported ever having TB or syphilis, respectively. 2 subjects (1.5%) were current smokers.

Table 9.6 Demographic, clinical and biochemical measurements for 135 subjects seen in the 2007 study and subsequently traced and assessed by the MDRS team in 2012. MAP = mean arterial pressure; FBS = Fasting blood sugar.

Characteristic	2007 visit	2012 visit
Age (years; median, IQR)	52.0 (45-58)	57.2 (48.3-64.1)
BMI (kg/m ² ; mean, SD)	29.4 (5.9)	27.4 (5.6)
Overweight (BMI>25 kg/m ²)	102 (75.6%)	80 (59.3%)
Duration of diabetes (yrs; med, IQR)	3.93 (2.2 – 7.8)	9.8 (8.2 – 13.6)
Hypertensive (see text)	94 (69.6%)	98 (72.6%)
sBP (mmHg; median, IQR)	130 (120-150)	142.5 (123-160)
dBp (mmHg; median, IQR)	80 (70-90)	81 (73-91)
MAP (mmHg; median, IQR)	96.7 (89.3 – 108.2)	103 (91-113.6)
HbA1c (NGSP %) (mean, SD)	9.4 (2.6)	8.3 (2.3)
FBS (mg/dL; mean, SD)	183.8 (88.0)	180.9 (90.1)
HIV reactive	15 (11.1%)	17 (12.6%)
Anaemia (WHO definition)	NA	26 (19.3%) 8M; 18F
Total cholesterol >5.0mmol/L	NA	40 (29.6%)
Total chol. (mmol/L; mean, SD)	NA	4.43 (1.12)
HDL cholesterol (mmol/L; mean, SD)	NA	1.12 (0.42)
LDL cholesterol (mmol/L; mean, SD)	NA	2.73 (0.90)
Triglycerides (mmol/L; mean, SD)	NA	1.41 (0.79)
Urine ACR (mg/mmol; mean; SD)	NA	22.48 (66.19)
Raised Urine ACR (n; %) [‡]	NA	53 (39.3%)
Urine dipstick	None 43; trace 44; 1+ 29; 2+ 6; 3+ 5; 4+ 1	NA
Serum creatinine (μmol/L;mean,SD)	83.2 (21.7)	85.1 (142.2)
Raised creatinine [†]	15 (11.1%)	16 (11.9%)

[†]Raised serum creatinine: men >110μmol/l; women >90μmol/l

[‡] Raised urine ACR: men >2.5 mg/mmol; women >3.5mg/mmol

9.4.5 Prevalence of grades of retinopathy

Prevalence of grades of retinopathy for 135 subjects seen in the 2007 study and subsequently traced and assessed by the MDRS team in 2012 are shown in Table 9.7. 2 subjects had received a course of laser treatment between the 2007 study and 2012: 1 subject received bilateral scatter laser and bilateral macular grid laser; 1 subject had received unilateral scatter laser.

Table 9.7 Prevalence with 95% CI of retinopathy grades according to worse eye in 135 persons with diabetes seen in the 2007 study and subsequently traced and assessed by the MDRS team in 2012. ST = sight threatening.

Grade (n; %, 95% CI)	2007	2012
No DR (level 10)	93 (68.9; 61.1-76.7)	49 (36.3; 28.2 – 44.4)
Any DR (level 20-71+)	40 (29.6; 21.9-37.3)	85 (63.0; 54.8 – 71.1)
Level 20	23 (17.0; 10.7 – 23.4)	41 (30.4; 22.6 – 38.1)
Level 30	9 (6.7; 2.5 – 10.9)	18 (13.3; 7.6 – 19.1)
Level 40	5 (3.7; 0.5-6.9)	16 (11.9; 6.4 – 17.3)
Level 50	0	2 (1.5; 0 – 3.5)
Proliferative (≥level 60+)	3 (2.2; 0 – 4.7)	8 (5.9; 1.9 – 9.9)
Ungradable	2 (1.5; 0 – 3.5)	0
ST maculopathy	12 (8.9; 4.1 – 13.7)	51 (37.8; 29.6 – 46.0)
STDR	17 (12.6; 7.0-18.2)	52 (38.5; 30.3 – 46.7)
No data	0	1 (0.7; 0 – 2.2)

9.4.6 Progression of grades of retinopathy

Of 135 subjects seen in 2012 two were classified as ‘ungradeable’ at baseline and 1 subject declined retinal examination in 2012. Therefore data for 2 visits was available for 132 subjects. Five year incidences of development of grades of retinopathy for subjects with level 10 (no retinopathy), level 20, level 30 and level 40 retinopathy at baseline are shown in Tables 9.8, 9.9, 9.10 and 9.11, respectively. Incidence of STDR ($p=0.0001$), ST maculopathy ($p=0.0001$) and PDR ($p=0.0001$)

increased with severity of baseline retinopathy (X^2 test for trend). Of 115 subjects without STDR at baseline (2007) 34 (29.6%; 21.3-37.9) had developed the condition at visit 2. Of 120 subjects without ST maculopathy at baseline 37 (30.8%) developed the condition by visit 2. Two (or more) step progression was observed in 48 subjects (36.4%; 95% CI 28.2-44.6); three (or more) step progression in 30 subjects (22.7%; 15.6-29.9). Figure 9.2 shows 5 year incidence of progression to sight threatening STDR, PDR and of 2 step and 3-step progression for subjects with level 10, level 20 and level 30 retinopathy at baseline.

Table 9.8 Five year incidence of development of all grades of DR, any DR, sight threatening maculopathy, STDR, 2-step progression and 3-step progression for 93 persons with level 10 (no retinopathy) at baseline. n =number of subjects reaching endpoint.

Grade progression	Number entering time interval	n	Incidence % (95% CI)
10 - 10	93	48	51.6 (41.5-61.8)
10 - 20	93	30	32.3 (22.8-41.8)
10 - 30	93	9	9.7 (3.7-15.7)
10 - 40	93	6	6.5 (1.5-11.5)
10 - 50	93	0	0
10 - 60+	93	0	0
10 - 20+ (any DR)	93	45	48.4 (38.2-58.5)
10 - ST Maculopathy	93	18	19.4 (11.3-27.4)
10-STDR	93	18	19.4 (11.3-27.4)
10-2+ step progression	93	30	32.3 (22.8-41.8)
10-3+ step progression	93	15	16.1 (8.7-23.6)

Table 9.9 Five year incidence of development of all grades of DR, sight threatening maculopathy, STDR, 2-step progression and 3-step progression for 23 persons with level 20 retinopathy at baseline. n =number of subjects reaching endpoint.

Grade progression	Number entering time interval	n	Incidence % (95% CI)
20 - 10	22	1	4.5 (0-13.3)
20 - 20	22	9	40.9 (20.4-61.5)
20 - 30	22	5	22.7 (5.2-40.2)
20 - 40	22	4	18.2 (2.1-34.3)
20 - 50	22	2	9.1 (0-21.1)
20 - 60+	22	1	4.5 (0-13.3)
20 - ST maculopathy	16	13	81.3 (62.1-100)
20 – STDR	16	13	81.3 (62.1-100)
20-2+ step progression	22	8	36.4 (16.3-56.5)
20-3+ step progression	22	7	31.8 (12.4-51.3)

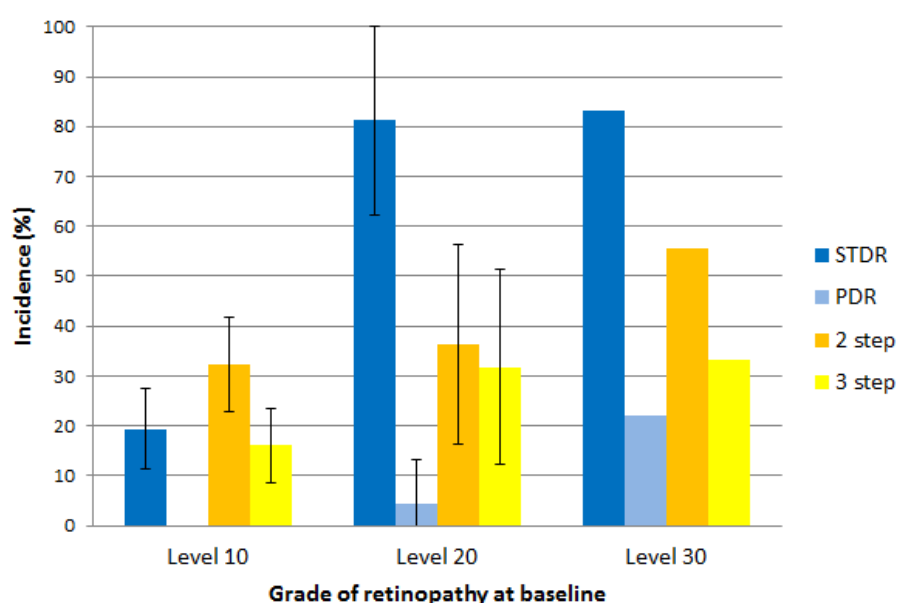
Table 9.10 Five year incidence of development of all grades of DR, sight threatening maculopathy, STDR, 2-step progression and 3-step progression for 9 persons with level 30 retinopathy at baseline. n =number of subjects reaching endpoint.

Grade progression	Number entering time interval	n	Incidence %
30 - 30	9	3	33
30 - 40	9	4	44
30 - 50	9	0	0
30 - 60+	9	2	22
30 - ST maculopathy	6	4	67
30 – STDR	6	5	83
30-2+ step progression	9	5	56
30-3+ step progression	9	3	33

Table 9.11 Five year incidence of development of proliferative DR (PDR), sight threatening maculopathy, 2-step progression and 3-step progression for 5 persons with level 40 retinopathy at baseline. n =number of subjects reaching endpoint.

Grade progression	Number entering time interval	n	Incidence %
40 - 60+ (PDR)	5	2	40
40 - ST maculopathy	5	4	80
40-2+ step progression	5	2	40
40-3+ step progression	5	2	40

Figure 9.2 Five year incidence of progression to sight threatening DR (STDR), proliferative DR (PDR) and of 2 step and 3-step progression for persons with diabetes and level 10 (n=93), level 20 (n=23) and level 30 DR (n=9) at baseline. Error bars indicate 95% CI.



9.4.7 Associations of progression of retinopathy

Higher mean glycosylated haemoglobin (HbA1c), longer duration of diabetes and lower haemoglobin were risk factors for progression of DR by 2 steps on the LDES scale in multivariate analysis (Table 9.12). In those subjects without STDR at baseline (n=115) baseline grade of DR was a risk factor for progression to STDR in

multivariate analysis (Table 9.13). Descriptive analysis showed that urine ACR did not demonstrate a linear association with probability of STDR; a log transformation (base 10) was more suitable for the model.

Table 9.12 Risk factors for association of 5 year progression of diabetic retinopathy by 2 steps on the LDES scale in 132 subjects with diabetes.

	OR	95% CI	p value
Univariate logistic regression			
Mean HbA1c (NGSP %)	1.37	1.13 - 1.66	0.001*
Duration (years)	1.05	0.99 – 1.12	0.096
Baseline grade of DR	1.51	1.05 – 2.17	0.026*
Type 1 diabetes	1.90	0.70 – 5.17	0.210
Mean sBP (mmHg)	1.00	0.92 – 1.34	0.872
Haemoglobin (g/dL)	0.86	0.72 – 1.02	0.086
Log[urine ACR] (mg/mmol)	1.28	0.83 – 1.95	0.261
LDL cholesterol (mmol/L)	0.91	0.61 -1.37	0.658
HDL cholesterol (mmol/L)	0.72	0.29 – 1.75	0.466
Triglycerides (mmol/L)	0.64	0.38 – 1.08	0.093
HIV positive	0.94	0.32 – 2.75	0.903
Age (years)	0.97	0.94 – 1.01	0.101
Sex (male)	0.70	0.32 - 1.54	0.378
Multivariate logistic regression			
Mean HbA1c (NGSP %)	1.47	1.13-1.90	0.004*
Duration (years)	1.08	1.00-1.17	0.040*
Haemoglobin (g/dL)	0.80	0.63-0.99	0.049*
Triglycerides (mmol/L)	0.55	0.29-1.04	0.065
Age (years)	0.96	0.92-1.01	0.081
Type 1 diabetes	0.35	0.08-1.48	0.153

Table 9.13 Risk factors for association of 5 year progression to sight threatening diabetic retinopathy (STDR) in 115 subjects with diabetes.

	OR	95% CI	p value
Univariate logistic regression			
Mean HbA1c (NGSP %)	1.22	1.01-1.49	0.044*
Baseline grade of DR	6.69	2.64-16.98	0.001*
Type 1 diabetes	3.21	1.06-9.72	0.039*
Duration of diabetes (years)	1.10	1.02-1.19	0.010*
Mean sBP (mmHg)	1.00	0.98-1.03	0.658
Haemoglobin (g/dL)	0.98	0.80-1.20	0.868
Log[urine ACR] (mg/mmol)	1.60	0.94-2.72	0.080
LDL cholesterol (mmol/L)	0.89	0.57-1.40	0.613
HDL cholesterol (mmol/L)	1.00	0.40-2.52	0.997
Triglycerides (mmol/L)	0.54	0.28-1.06	0.075
HIV positive	0.98	0.28-3.44	0.979
Age (years)	0.97	0.94-1.01	0.116
Sex (male)	1.07	0.45-2.53	0.875
Multivariate logistic regression			
Baseline grade of DR	6.62	2.32-18.87	0.001*
Triglycerides (mmol/L)	0.53	0.23-1.21	0.129
Mean HbA1c (NGSP %)	1.16	0.91-1.47	0.241

9.4.8 Vision

For the purpose of this analysis visual acuity scores from 2007 (corrected Snellen acuities in the better eye) were converted to ETDRS letter scores using a standard conversion table [337]. Comparison was then made with 2012 ETDRS letters measurement in the better eye. (The eye with better vision in 2007 may not have been the same as the eye with better vision in 2012). Visual acuity measurements for 135 subjects seen in the 2007 study and subsequently traced and assessed by the MDRS team in 2012 are shown in Table 9.14. In 2012 according to WHO definitions [310] 129 subjects (95.6 %; 95% CI 92.1 - 99.0) had 'normal vision' (equal to or better than 60 letters), 3 subjects (2.2 %; 0-4.7) had 'moderate visual

impairment' (50 to 59 letters), and 3 subjects (2.2 %; 0-4.7) were 'severely visually impaired or blind' (<50 letters). In 2012 the most common primary causes of visual impairment for subjects with corrected visual acuity worse than 80 letters (equivalent to 6/12 Snellen or worse) (n=53) were DR (43%) cataract (22%), and both DR and cataract (21%) (Table 9.15). Therefore in 64% of cases DR was the sole or equal contributing cause of visual loss.

Over the follow-up period 25 subjects (18.8%) lost 5 or more ETDRS letters of which 7 subjects (5.3%) lost 15 or more letters. Of these 25 subjects 2 (1.5%) progressed to moderate visual impairment (50-59 letters) and 3 (2.3%) became 'severely visually impaired or blind' (<50 letters). The most common primary causes of visual loss for the 25 subjects who lost five or more letters were DR (36%) cataract (28%), and both DR and cataract (20%) (Table 9.16). Therefore in 56% of cases DR was the sole or equal contributing cause of visual loss. In univariate analysis loss of 5 or more ETDRS letters was associated with presence of STDR at follow up visit (OR 3.29, 95% CI 1.32-8.18, p=0.010) but not age (OR 1.03, 0.99-1.072, p=0.179) or duration of diabetes (OR 1.03, 0.96-1.10, p=0.389).

Table 9.14 Prevalence with 95% CI of corrected ETDRS visual acuities according to better eye in 135 persons with diabetes seen in the 2007 study and subsequently traced and assessed by the MDRS team in 2012. Approximate Snellen acuities in parentheses.

Visual acuity (ETDRS letters)	2007		2012	
	n	% (95% CI)	n	% (95% CI)
≥ 90 (6/5)	8	5.9 (2.0-9.9)	13	9.6 (4.7-14.6)
80 - 89 (6/7.5)	37	27.4 (19.9-34.9)	69	51.1 (42.7-59.5)
70 - 79 (6/12)	67	49.6 (41.2-58.1)	31	23.0 (15.9-30.1)
60 - 69 (6/18)	15	11.1 (5.8-16.4)	16	11.9 (6.4-17.3)
50 - 59 (6/30)	3	2.2 (0-4.7)	3	2.2 (0-4.7)
40 - 49 (6/75)	0		1	0.7 (0-2.2)
Hand Movements	3	2.2 (0-4.7)	2	1.5 (0-3.5)
Light Perception	0		0	
No light perception	0		0	
No data	2	1.5 (0-3.5)	0	

Table 9.15 Primary causes of visual impairment (VI) in the opinion of the examining clinician at 2012 visit for subjects with diabetes and corrected visual acuity worse than 80 letters. Subjects classified according to level of visual impairment (n=53). Approximate snellen equivalents: 70-79 letters = 6/12; 60-69 = 6/18; 50-59 = 6/24 'Moderate visual impairment'; <50 letters = 6/36 or worse 'Severely visually impaired or blind'.

Primary cause of VI	Level of visual impairment (ETDRS letters)				Total
	70-79	60-69	50-59	<50	
n	31	16	3	3	53
DR	14 (45%)	8 (50%)		1	23 (43%)
DR and cataract	3 (10%)	4 (25%)	3	1	11 (21%)
Cataract	10 (32%)	3 (19%)			13 (24%)
AMD	3 (10%)	0			3 (6%)
Glaucoma	0	1 (6%)		1	2 (4%)
Other	1 (3%)*	0			1 (2%)

* 1 subject PCO

Table 9.16 Primary causes of visual loss between 2007 and 2012 visits in the opinion of the examining clinician for subjects with diabetes and loss of 5 or more letters. Subjects classified according to number of letters lost and level of visual impairment (n=25).

Primary cause of visual loss	Number of ETDRS letters lost			Level of visual impairment	
	5-14	≥15	Total	Progression to Moderate VI*	Progression to Severe VI†
n	18	7	25	2	3
DR	6	3	9 (36%)		1
DR and cataract	4	1	5 (20%)	2	1
Cataract	6	1	7 (28%)		
AMD	2		2 (8%)		
Glaucoma		2	2 (8%)		1

* 'Moderate visual impairment' = 50-59 letters (equivalent to 6/24 Snellen)

† 'Severely visually impaired or blind' = <50 letters (equivalent to 6/36 or worse)

9.4.9 MDRS 6 and 7 year progression data

Of the 2007 cohort, 41 subjects were recruited (by systematic random sampling from the QECH diabetes clinic) into the main MDRS 24 month cohort study (2012; Chapters 6, 7 and 8). Of these 41 subjects 38 were seen at MDRS visit 2 (2013) and 36 were seen at MDRS visit 3 (2014) providing 6 and 7 year longitudinal data. 2 subjects died by visit 2 and a further 1 by visit 3. Baseline demographic, clinical and biochemical measurements for 281 subjects included in the 2007 study categorised by inclusion in the main MDRS cohort are shown in Table 9.17. Prevalence of retinopathy grades in the 41 subjects seen in 2007 and subsequently recruited to the MDRS main cohort are shown in Table 9.18.

Cumulative incidence at 5, 6 and 7 years of development of grades of retinopathy for subjects with level 10 (no retinopathy) and level 20 DR at baseline are shown in Tables 9.19 and 9.20, respectively. Of 3 subjects with level 30 DR at baseline (2007), by 7 years 2 had developed PDR and 1 developed level 50 DR; all developed ST maculopathy. The 1 subject with level 40 DR at baseline had level 70 DR at 5 years and died before year 6. The 1 subject with Level 60 at baseline at baseline had Level 60 DR at 5 years, received bilateral scatter and macular laser and had level 20 DR, level 4 maculopathy at 6 years. Further macular laser was performed after visit 2. The subject subsequently moved to Lilongwe and did not attend at 7 years. Of 34 subjects without STDR at baseline (2007) and seen at 7 years 12 (35.3%; 19.2-51.4) had developed the condition at 7 years (of these 11 had ST maculopathy). During the course of the study 8 subjects were listed for scatter and macular laser (8 started a course of treatment; 7 completed the course), 5 for scatter alone (5 started a course of treatment; 4 completed the course) and 2 for macular laser alone (both completed the course).

Table 9.17 Analysis of bias: baseline demographic, clinical and biochemical measurements for 281 subjects with diabetes included in the 2007 cohort study. Subjects categorised by inclusion in the main MDRS cohort (2012-2014).

Characteristic	Subjects not included in MDRS main cohort	Subjects included in MDRS main cohort	P value
n	240	41	
Female sex	164 (68.3%)	26 (63.4%)	0.589 \neq
Age (yrs, median, IQR)	55 (46-63)	52 (42-57)	0.107 \dagger
Type 1 diabetes	28 (11.7%)	4 (9.8%)	0.999 \neq
BMI (kg/m ² ; mean, SD)	28.5 (6.1)	28.9 (6.2)	0.699 \dagger
Duration (at baseline) (yrs, med, IQR)	5.0 (2.2-8.9)	4.0 (1.1-10.8)	0.549 J
sBP (mmHg; med, IQR)	140 (120-155)	130 (128-140)	0.324 \dagger
dBp (mmHg; med, IQR)	80 (70-90)	80 (70-90)	0.640 \dagger
HbA1c (NSGP%;mean,SD)	9.3 (2.3)	9.9 (3.0)	0.1424 \dagger
HIV positive	27 (11.3%)	3 (7.3%)	0.590 \neq
Urine dipstick	None 59; trace 72; 1+ 45; 2+ 16; 3+ 7; 4+ 4	None 13; trace 14; 1+ 9; 2+ 1; 3+ 4; 4+ 0	0.894 χ
Serum creatinine \ddagger	0.97 (0.30)	0.92 (0.32)	0.368 \dagger
Raised serum creatinine \dagger	40 (16.7%)	3 (7.3%)	0.160 \neq

\dagger Raised serum creatinine: men >110 μ mol/l; women >90 μ mol/l

\ddagger Serum creatinine (mg/dl, mean, SD)

\neq Fisher's exact test; \dagger Unpaired t-test; J Wilcoxon rank sum; χ χ^2 test for trend

Table 9.18 Prevalence with 95% CI of retinopathy grades according to worse eye in 41 persons with diabetes seen at 4 assessments: 2007 (original study of diabetes complications), 2012 (MDRS visit 1), 2013 (MDRS visit 2) and 2014 (MDRS visit 3). STM =sight threatening maculopathy.

Grade (n; %; 95% CI)	Study Visit			
	2007	2012	2013	2014
n	41	41	38	36
10	27 (65.9, 51.4-80.4)	13 (31.7, 17.5-45.9)	15 (39.5, 24.0-55.0)	10 (27.8, 13.2-42.4)
20	7 (17.1, 5.6-28.6)	13 (31.7, 17.5-45.9)	7 (18.4, 6.1-30.7)	13 (36.1, 20.4-51.8)
30	3 (7.3, 0-15.3)	6 (14.6, 3.8-25.4)	7 (18.4, 6.1-30.7)	4 (11.1, 0.8-21.4)
40	1 (2.4, 0-7.1)	4 (9.8, 0.7-18.9)	4 (10.5, 0.8-20.3)	4 (11.1, 0.8-21.4)
50	0	1 (2.4, 0-7.1)	1 (2.6, 0-7.7)	1 (2.8, 0-8.2)
60+	1 (2.4, 0-7.1)	4 (9.8, 0.7-18.9)	4 (10.5, 0.8-20.3)	4 (11.1, 0.8-21.4)
Any DR	12 (29.3, 15.4-43.2)	28 (68.3, 54.1-82.5)	23 (60.5, 45.0-76.0)	26 (72.2, 57.6-86.8)
STM	3 (7.3, 0-15.3)	16 (39.0, 24.1-53.9)	18 (47.4, 31.5-63.3)	12 (33.3, 17.9-48.7)
STDR	4 (9.8, 0.7-18.9)	17 (41.5, 26.4-56.6)	18 (47.4, 31.5-63.3)	14 (38.9, 23.0-54.8)
Ungrade- able	2 (4.9, 0-11.5)	0	0	0

Table 9.19 Cumulative incidence at 5, 6 and 7 years of development of any retinopathy, level 30 DR, level 40 DR, level 60+ DR, sight threatening maculopathy and STDR for 27 persons with diabetes and level 10 (no retinopathy) at baseline.

	Any retinopathy				Level 30			
T	N	n	C. Inc.	95% CI	N	n	C. Inc.	95% CI
5	27	14	51.9	0-13.6	27	1	3.70	0-10.8
6	13	1	55.5	0-14.2	26	3	15.0	1.4-28.6
7	12	3	67.2	0-16.3	22	0	15.0	1.1-28.9

	Level 40				Level 60+			
T	N	n	C. Inc.	95% CI	N	n	C. Inc.	95% CI
5	27	2	7.4	0-17.3	27	0	0	
6	25	1	11.2	0-23.2	27	2	7.6	0-17.7
7	23	0	11.2	0-23.4	24	0	7.6	0-17.9

	ST maculopathy				STDR			
T	N	n	C.Inc.	95% CI	N	n	C. Inc.	95% CI
5	27	5	18.5	3.9-33.2	27	5	18.5	3.9-33.2
6	22	2	26.1	9.4-42.8	22	2	26.1	9.4-42.8
7	19	1	30.1	12.3-47.9	19	1	30.1	12.3-47.9

T = time from recruitment (years); N = number entering time interval; n = new cases diagnosed during year; C. inc. = cumulative incidence (%); CI = confidence interval; ST = sight threatening; STDR = sight threatening diabetic retinopathy

Table 9.20 Cumulative incidence at 5, 6 and 7 years of development of level 60+ DR, sight threatening maculopathy and STDR for 7 subjects with diabetes and level 20 DR at baseline.

	Level 60+			ST maculopathy‡			STDR‡		
T	N	n	C. Inc.	N	n	C. Inc.	N	n	C. Inc.
5	7	1	14.3	6	4	66.7	6	4	66.7
6	6	1	28.6	2	1	83.3	2	1	83.3
7	5	1	42.9	1	1	100	1	1	100

T = time from recruitment (years); N = number entering time interval; n = new cases diagnosed during year; C. inc. = cumulative incidence (%); CI = confidence interval; ST = sight threatening; STDR = sight threatening diabetic retinopathy. ‡ - those with sight threatening maculopathy/STDR at baseline omitted from analysis

9.5 Discussion

9.5.1 Principal findings

This chapter details progression of grades of retinopathy and visual impairment over 5 years in a cohort of people with diabetes from Southern Malawi who were not treated with laser. In 135 subjects (48.0% of the original 281 subject cohort) prevalence of any retinopathy, STDR and PDR increased from 29.6% (95% CI 21.9-37.3) to 63.0% (54.8 – 71.1), 12.6% (7.0-18.2) to 38.5% (30.3-46.7) and from 2.2% (0 – 4.7) to 5.9% (1.9 – 9.9), respectively. Five year incidence of any DR in those without evidence of retinopathy at baseline was 48.4% (38.2-58.5). The five year incidence of STDR for those with level 10 and level 20 retinopathy at baseline was 19.4% (11.3-27.4) and 81.3% (62.1-100), respectively. The five year incidence of PDR for those with level 10, level 20, level 30 and level 40 retinopathy at baseline was 0%, 4.5%, 22% and 40%, respectively. Higher glycosylated haemoglobin (HbA1c), longer duration of diabetes and lower haemoglobin level were risk factors for progression of retinopathy in multivariate analysis. Over the follow-up period 25 subjects (18.8%) lost 5 or more ETDRS letters of which 7 subjects (5.3%) lost 15 or more ETDRS letters. 2 subjects (1.5%) progressed to moderate visual impairment (50-59 letters) and 3 (2.3%) became 'severely visually impaired or blind' (<50 letters). In 56% of cases DR was the sole or equal contributing cause of visual loss.

9.5.2 Strengths and weaknesses of this work

This work, along with the main MDRS cohort, represents the first cohort study of DR from Sub-Saharan Africa. Strengths of the work include a systematic approach to subject tracing, a robust procedure for grading retinopathy and the large number of systemic parameters which were measured. Subject follow-up in this region is extremely challenging. The 48% follow-up rate (plus a further 5% confirmed dead) is a considerable achievement. However, this is a limitation of the work; conclusions drawn from our work should be generalised with caution.

In this study the procedures for assessing retinopathy and vision differed between the baseline and final assessments. Detection of retinopathy and maculopathy by photographic grading is quoted as being 89–93% sensitive and 86–94% specific judged against the standard of a consultant specialist in medical retinal disease [94,95]. However, dual grading of retinal photographs at a recognised reading centre is now considered the reference standard for grading. The more robust assessment procedure used in 2012 could have detected a greater amount of disease. It is unlikely that this factor would substantially affect the results of this study. There is evidence of differences between measures of visual acuity using ETDRS and Snellen charts. ETDRS measurements yield better VA and differences are more marked in persons with low vision [338-340]. The effect of these differences in this study would be to reduce the degree of visual loss recorded.

9.5.3 Analysis of bias

It is important to assess to what extent the subjects who were traced and assessed in 2012 are representative of the whole cohort. While 15 subjects (5.3%) were confirmed dead the actual number of deaths is likely to be far higher. Differences between the 'seen' and 'not seen' groups may be predictors of mortality. Regarding baseline demographic and clinical parameters, subjects not seen in 2012 were older and had higher blood pressure than those who were traced and assessed. A higher prevalence of raised serum creatinine and STDR in the group who were not seen in 2012 suggests a higher baseline prevalence of microvascular complications of diabetes. Vision was worse in the 'not seen' group. This may reflect a greater prevalence of STDR and cataract (for which age is a risk factor). Poor vision may be a risk factor for mortality either as a marker of microvascular damage in diabetes or as a marker of age. Alternatively poor vision may directly affect an individual's ability to survive in Southern Malawi.

9.5.4 Comparison with African studies

Few cohort studies from the African continent are available for comparison.

Chapter 4, Section 4.4.4 details the only published studies which are summarised in

Table 4.4. In Mauritius 1998 researchers followed up a population based study performed in 1992 [212] with a survey of diabetes complications [215]. Of subjects with diabetes in the initial survey 40.5% were re-examined. The 6 year incidence of DR and PDR in subjects with diabetes but no DR in the first survey was 23.8% (95% CI 18.3-29.3) and 0.4% (0-1.2), respectively. The 5 year incidence of any DR in our study (48.4% (38.2-58.5)) was higher.

In Mauritius the 6 year incidence of PDR in subjects with mild non-proliferative diabetic retinopathy (NPDR) (equivalent to level 20 in LDES grading) was 5.2% (0-10.9). For subjects with moderate NPDR (equivalent to LDES level 30 or level 40) the incidence of PDR was 29.4% (7.7-51.1). These figures are similar to the five year incidences of PDR in our study (level 20 4.5%; level 30 22%; level 40 40%). Duration of diabetes and fasting blood glucose were independently associated with incidence of retinopathy. Differences between the two studies are likely to reflect multiple disparities between Mauritius and Southern Malawi including ethnicity, access to health services and prevalence of comorbidities.

Although there are few prospective cohort studies from Africa, studies reflecting cumulative incidence of DR are available. In South Africa, Distiller et al [237] reported on 1520 type 1 and 8026 type 2 persons who had maintained membership for ≥ 5 years of a community-based, privately funded diabetes management program. In type 1 subjects prevalence of any retinopathy at baseline and at 5 years was 22.3% and 28%, respectively and in type 2 subjects 20.5% and 26.6%, respectively. These are much smaller increases in prevalence of any DR than shown in the present study: from 29.6% (95% CI 21.9-37.3) to 63.0% (54.8 – 71.1) in 5 years. In large part this is likely to reflect better access to health services in the South African cohort.

9.5.5 Comparison with studies in Europe and North America

High quality prospective cohort studies of DR are available from Europe and North America. Table 9.21 summarises selected studies reporting incidence and progression of diabetic retinopathy. The Wisconsin Epidemiological study of

Diabetic Retinopathy (WESDR) was a population based epidemiological study performed in the 1980s. For subjects with diabetes diagnosed at 30 years of age or older who had no retinopathy at baseline 4 year incidence of any retinopathy for insulin users and non- users of insulin was 47% and 34%, respectively [83]. Overall incidence of PDR in insulin users and non-users of insulin was 7% and 2%, respectively. For those with diabetes diagnosed at less than 30 years and no retinopathy at baseline 4 year incidence of retinopathy was 59% [84]. Overall 4 year incidence of PDR was 11%.

Younis et al [9] reported data from 4770 persons with type 2 diabetes registered with general practices in one English city who had two or more screening events as part of the Liverpool Diabetic Eye Study (LDES). Five year cumulative incidence of any DR in those without evidence of retinopathy at baseline was 30.5% (28.2–32.8) compared to 48.4% (38.2–58.5) in the present study. The five year cumulative incidence of STDR for those with level 10, level 20 and level 30 retinopathy at baseline was 3.9% (2.8–5.0), 28.9 (24.4–33.4) and 63.2 (53.4–73.0), respectively. The corresponding figures in our study were much higher: 19.4% (11.3–27.4), 81.3% (62.1–100) and 83%, respectively. As in the present study longer duration of diabetes was associated with progression to STDR. The LDES included a smaller number of people with type 1 diabetes. Younis et al [96] reported data from 501 subjects. The five year cumulative incidence of any DR in those without evidence of retinopathy at baseline was 36.8% (29.6–44.1). The five year cumulative incidence of STDR for those with level 10, level 20 and level 30 retinopathy at baseline was 3.9% (1.4–5.4), 26.8% (13.6–40.0) and 66.6% (36.9–95.7), respectively.

More recently Jones et al [331] reported data on 20,686 persons with type 2 diabetes seen in the county of Norfolk as part of the English national screening program. Among subjects without retinopathy at baseline, cumulative incidence at 5 years of background retinopathy (equivalent to LDES level 20) was 35.9% (34.8–37.0), pre-proliferative retinopathy (equivalent to level 40) was 4.0% (3.5–4.4), sight threatening maculopathy 0.59% (0.4–0.8), and PDR 0.68% (0.51–0.90). Among those with background retinopathy at baseline, after 5 years 23.0% (20.7–25.6) developed

preproliferative retinopathy, 5.2% (4.05-6.67) developed sight threatening maculopathy, and 6.1% (4.85-7.66) developed PDR. Therefore for subjects with background retinopathy (equivalent to LDES level 20) at baseline the rate of progression to STDR was lower than the present study, however, the incidence of PDR was slightly higher.

In Wales, Thomas et al [336] reported incidence of any DR and referable DR (equivalent to LDES level 40, or exudate or thickening within 1 disc diameter (DD) of the centre of the fovea, or circinate or group of exudates within the macula, or any microaneurysm or haemorrhage within 1DD of the centre of the fovea only if associated with a best VA of $\leq 6/12$. i.e. roughly equivalent to STDR as defined in the present study) in persons with type 2 diabetes attending an annual screening service and whose first screening episode indicated no evidence of retinopathy. After four years the cumulative incidence of any DR and referable DR was 36.0% and 1.2%, respectively.

While not designed as observational cohort studies, a number of large randomised trials have reported incidence and progression rates of DR. The United Kingdom Prospective Diabetes Study (UKPDS) [13] reported data from 1919 persons with type 2 diabetes. Of 1216 with no retinopathy at baseline 41% developed at least 1 microaneurysm by 6 years and 22% developed microaneurysms in both eyes or worse. Of 703 subjects with any DR at baseline 29% progressed by 2 or more steps on the ETDRS scale. Development of retinopathy (incidence) was associated with baseline glycaemia, glycaemic exposure over 6 years, high blood pressure and with not smoking. In those who already had retinopathy, progression was associated with older age, male sex, higher HbA1c and with not smoking.

In summary, compared to recent European studies 5 year incidence of any retinopathy in our study was higher: 48.4% (38.2-58.5) vs estimates between 30.5% [9] and 40.6% [13,331]. In our study 5 year progression to STDR from no DR (level 10) was approximately 5 times that reported in recent European studies: 19.4% (11.3-27.4) vs estimates between 3.9% [9,96] and 4.0% [331]. Five year progression

to STDR from background DR (level 20) was approximately 3 times higher: 81.3% (62.1-100) vs estimates between 26.8 [96] and 28.9 [9]. Five year progression to PDR from no DR (level 10) and background DR (level 20) was similar in our study to recent studies from England [331] and Mauritius [215]. Differences between figures from our study and recent European work are likely to reflect multiple disparities between populations including ethnicity, access to health services and presence of comorbidities including poorly controlled hypertension and infective disease. Comparisons between studies must be made with caution in view of different study designs and different follow-up rates. Of particular note mortality rates are likely to differ greatly between populations and are an important cause of data censoring.

Table 9.21 Summary table of selected studies reporting incidence and progression of diabetic retinopathy.

Study	WESDR [23]	WESDR [22]	Wales [20]	UKPDS [19]	LDERS [18]	LDERS [17]	Norfolk [21]	MDRS	Mauritius [15]
Dates	1979-1986	1979-1986	2005-2009	1977-1991	1991-1999	1991-1999	1990-2006	2007-2012	1992-1998
Type of study	Primary care based cohort		Retrospective analysis of DR screening prog.	RCT	Prospective study of DR screening programme		Retrospective analysis of DR screening	Primary care based cohort	Population-based cohort
Subjects	Diagnosis <30yrs	Diagnosis ≥30yrs	Type 2 diabetes	Type 2 diabetes	Type 1 diabetes	Type 2 diabetes	Type 2 diabetes	All subjects with diabetes	All subjects with diabetes
Follow-up(yrs)	4	4	4	5	5	5	5	5	6
Incidence of any DR (%) †	59	34 {no insulin} 47 {insulin}	36.0 (35.3-36.6)	40.6 (37.9-43.4)	36.8 (29.6–44.1)	30.5 (28.2–32.8)	40.6	48.4 (38.2-58.5)	23.8 (18.3-29.3)
Incidence of STDR (%)			1.2 {L10}		3.9 {L10} 26.8 {L20} 66.6 {L30}	3.9 {L10} 28.9 {L20} 63.2 {L30}	4.0 {L10}* 23.0 {L20}*	19.4 {L10} 81.3 {L20} 83.0 {L30}	
Incidence of PDR (%)	11	2 {no insulin} 7 {insulin}					0.68 {L10} 6.1 {L20}	0 {L10} 4.5 {L20} 22 {L30} 40 {L40}	0.4 {10} 5.2 {20} 29.4 {30/40}

{ } Subgroup or baseline LDES retinopathy grade: L10 = level 10, L20 = level 20 etc. +95% confidence interval in parentheses. * Incidence of Level 40 retinopathy only (sight threatening maculopathy data not included). WESDR = Wisconsin epidemiological study of diabetic retinopathy; MDRS = Malawi diabetic retinopathy study; LDES = Liverpool diabetic eye study; UKPDS = United kingdom prospective diabetes study; RCT = Randomised controlled trial; DR Diabetic retinopathy; STDR = Sight threatening diabetic retinopathy; PDR = Proliferative diabetic retinopathy.

9.5.6 Vision

Interestingly in our study the overall levels of ‘moderate visual impairment’ (50 to 59 letters) and ‘severe visual impairment or blindness’ (<50 letters) did not change between 2007 and 2012. The proportion of subjects with VA <70 letters (equivalent to 6/12 Snellen) rose from 15.5% to 16.3%. 18.8% of subjects lost 5 or more ETDRS letters, 5.3% lost 15 or more letters, 2 subjects (1.5%) progressed to moderate visual impairment (50-59 letters) and 3 (2.3%) became ‘severely visually impaired or blind’ (<50 letters). Clearly some subjects recorded better VA in 2012 than in 2007. Explanations for this include medical interventions including cataract surgery. An important confounder is the difference in methods of measurement of visual acuity in 2007 and 2012 discussed above (Section 9.5.2). A potential bias is that subjects who became visually impaired may have been less likely to attend the follow-up visit in 2012. Visual impairment may increase the chance of mortality in a society where loss of vision entails loss of economic productivity. Together these factors may explain why a greater degree a visual impairment was not seen in this cohort study.

Few studies reporting visual acuity in subjects with diabetes in Africa are available for comparison with our study. Published studies are summarised in Chapter 4, Section 4.4.6 of this thesis. The Diabetes in Egypt project [246] reported VA in 427 subjects with diabetes. Of these 31 (7.3 %) were blind (defined as BCVA in the better eye less than 6/60). Prevalence of visual impairment (VA 6/60 or worse in the better eye) in the MDRS main cohort at baseline (Chapter 6) and the present study was 1.4% and 2.2%, respectively. The population-based Mauritius diabetes complication study [212] reported best correct visual acuity (BCVA) worse than 6/12 in 7.1% of subjects with diabetes at baseline. The corresponding figures in the MDRS main cohort at baseline (Chapter 6), the present study in 2007 and the present study in 2012 are 7.3%, 15.5% and 16.3%, respectively. Unfortunately no visual acuity data was reported from the follow-up assessment in the Mauritius study [215]. Indeed, to our knowledge, no cohort studies of DR from Africa have reported VA data except at baseline.

Prevalence of visual impairment in subjects with diabetes has been reported in a number of studies from Europe and North America. In the WESDR a VA of 6/60 or worse in the better eye was reported at baseline in 3.6% of type 1 subjects and 1.6% of type 2 subjects [10,11]. In Iceland Kristinsson et al. [290] reported VA of 6/60 or worse in the better eye in 1.0% of type 1 subjects and 1.6% of type 2 subjects. Corresponding figures in the MDRS are 1.4% in the main cohort at baseline (Chapter 6) and 2.2% in the present study. Incidence of visual impairment (VI) was reported for persons with type 1 diabetes in the WESDR [85]. During the first 4 years of follow-up the annual incidence of VI (defined as best-corrected VA in the better eye of 6/12 or worse) and severe visual impairment (6/60 or worse) was 0.4% and 1.2%, respectively. Increased risk of VI was associated with more severe baseline retinopathy, presence of cataract, higher HbA1c, presence of hypertension and current smoking, but not proteinuria.

In the UKPDS [341] the percentage of subjects with VA worse than 0.3 LogMAR (minimum angle of resolution)(equivalent to 6/12 Snellen) at baseline in the 'tight blood pressure control' group and the 'less tight BP control' group was 1.7% and 1.8%, respectively. After 6 years the figures were 6.4% and 5.9%, respectively. In our study the proportion of subjects with VA <70 letters (equivalent to 6/12 Snellen) was 15.5% in 2007 and 16.3% in 2012. Hall et al [335] studied blind registrations attributed to DR in Scotland. These authors estimated that the annual incidence of blindness (defined as visual acuity in the better eye below 3/60) in the population with diabetes was 0.04%.

Our results can also be compared to population-based (i.e. not confined to persons with diabetes) cohort studies reporting incidence of visual impairment. The Beaver Dam eye study [342] reported an overall incidence of VI (VA worse than 6/12 in the better eye) between examinations (5-year interval) of 1.4% (varying from 0.1% in persons 50–54 years of age to 14.6% in those 85 years of age and older). The 5 year incidence of severe VI (VA<6/60) was 0.4% (varying from 0.0% in persons 50–54 years of age to 6.9% in those ≥ 85 years of age).

9.6 Chapter summary

This chapter provides an estimate of incidence and progression of grades of retinopathy over 5 years in a cohort of persons with diabetes in Southern Malawi. In this cohort 5 year progression to STDR from 'no DR' and 'background DR' was approximately 5 times and 3 times that reported in recent European studies, respectively. Higher glycosylated haemoglobin and lower haemoglobin level were risk factors for progression of retinopathy. I have reported prevalence and incidence of visual impairment in this cohort. The vast majority of subjects did not have access to laser treatment during the 5 year follow-up period. Data on progression of DR and incidence of visual impairment in an untreated cohort provides a baseline against which future studies can judge efficacy and cost effectiveness of laser treatment in this population.

Chapter 10. Case-Control Study of Endothelial Function in Malawian Subjects with Diabetes

10.1 Aims of the chapter

This chapter details the results of a case-control study of endothelial function performed in a sub-group of the Malawi Diabetic Retinopathy Study (MDRS) cohort and a group of Malawian subjects without diabetes.

10.2 Introduction

Endothelial dysfunction is implicated in the pathophysiology of diabetic retinopathy (DR). Both diabetes and its complications are associated with altered serum levels of biomarkers of endothelial dysfunction and decreased *in vivo* responsiveness of the peripheral vascular endothelium. This subject is reviewed in Chapter 1, Sections 1.5.4 and 1.5.5. In Malawi a number of factors, including high levels of infective disease, may modify the local and systemic response to hyperglycaemia and result in dysfunction of the vascular endothelium. In this nested case-control study I aimed to characterise endothelial function in Malawian subjects with diabetes and investigate relationships with severity of retinopathy. In this chapter I report the results of these investigations. Firstly in comparison to baseline cross-sectional DR data and secondly in relation to progression of DR at 24 months.

10.3 Methods

10.3.1 Subjects and assessment

Study setting, sampling of subjects, clinical assessment, assessment of retinopathy, measurement of serum markers of endothelial dysfunction and pulse amplitude tonometry are fully described in Chapter 5, Methods. In brief, a subset of subjects from the main cohort study (described in Chapter 5, Section 5.9) were investigated

plus subjects without diabetes. I studied endothelial function in 4 groups each consisting of a minimum of 40 subjects:

1. subjects without diabetes
2. subjects with diabetes but without DR at baseline
3. subjects with diabetes and DR but without sight threatening diabetic retinopathy (STDR)
4. subjects with diabetes and STDR

All subjects recruited to the main cohort study were offered the chance to participate in the case control study until each of the above groups reached their recruitment target. Inclusion and exclusion criteria were the same as the main cohort study. Inclusion criterion: diagnosis of diabetes according to American Diabetes Association (ADA) criteria [123] (Chapter 5, Box 5.1). Exclusion criteria: age <18 years; first visit to the diabetes clinic, residence >60km from the hospital in question; diagnosis of gestational diabetes according to ADA criteria [123].

Systematic random sampling was used to recruit control subjects (without diabetes) from spouses of patients attending the QECH diabetes clinic (see Chapter 5, Section 5.11.3). Inclusion criterion was being a spouse of a patient attending the QECH diabetes clinic. Exclusion criteria were: age <18 years and diagnosis of diabetes or gestational diabetes according to ADA criteria. Therefore control subjects with fasting blood glucose $\geq 7.0\text{mmol/l}$ or HbA1c $\geq 6.5\%$ were excluded from the study.

10.3.2 Statistical analysis

General statistical methods are described in Chapter 5, Section 5.9.10. Details of specific analyses used in the endothelial sub-study are given in Chapter 5, Section 5.11.6. Briefly, endothelial function was compared across 4 groups (described above) applying multiple linear regression. I defined 3 binary variables, namely presence of diabetes (yes/no), presence of diabetic retinopathy (yes/no) and presence of STDR (yes/no). These factors are nested, for example, absence of diabetes implies absence of DR and STDR, and absence of DR implies absence of

STDR. The multiple regression model was constructed taking into account this nested design and the factors included in the model were diabetes and the interaction terms diabetes*DR and diabetes*DR*STDR. Several outcome variables were analysed separately using multiple regression models, these include five serum markers: C-reactive protein (CRP), Inter cellular adhesion molecule 1 (ICAM-1), E-selectin, vascular cell adhesion molecule 1 (VCAM-1) and Vascular endothelial growth factor (VEGF), in addition to the Reactive Hyperaemia Peripheral Arterial Tonometry (rhPAT) index, Framingham reactive hyperaemia index (FRHI) and augmentation index (AI). Additionally, I constructed a logistic regression model (backwards stepwise with probability of removal of 0.2) to investigate the association between presence of diabetes and the following variables: age, sex, CRP, VEGF, ICAM-1, VCAM-1, E-selectin, and rhPAT index. Odds ratio (OR) and the corresponding 95% CIs were provided.

Baseline endothelial function was compared between subjects with diabetes whose retinopathy progressed by 2 steps on the LDES scale and subjects whose retinopathy did not progress at 24 months. Baseline endothelial function was also compared between subjects who had died and those who survived at 24 months. Multiple logistic regression analysis was used to analyse the effect on progression of retinopathy (and, in a separate analysis, on death) of an initial 8 variables: age, sex, CRP, VEGF, ICAM-1, VCAM-1, E-selectin, and rhPAT index. Variables including HbA1c and sBP could be considered to be on the causative pathway between the 'exposure' (markers of endothelial dysfunction) and the 'outcomes' (progression of retinopathy or death). These variables therefore did not meet the definition of confounders and were not included in the models.

10.4 Results: analysis of baseline data

10.4.1 Participants

163 subjects from the MDRS main cohort study were approached to participate in the endothelial sub-study. 24 declined; 139 were included. 57 spouses of patients attending the QECH diabetes clinic were approached to participate as controls. 15 declined and 2 were excluded due to raised FBS and/or HbA1c; 40 were included. Table 10.1 shows the baseline demographic, clinical and biochemical characteristics of included subjects.

Table 10.1 Baseline demographic, clinical and biochemical characteristics of 179 subjects included in the MDRS sub-study of endothelial function. Subjects categorised by diabetes status and grade of retinopathy: 1=no diabetes (control subjects), 2=diabetes no retinopathy, 3=diabetic retinopathy (DR), 4=sight threatening diabetic retinopathy (STDR).

Group	1. Control	2. No DR	3. DR	4. STDR	p ≠
n	40	53	43	43	
Age (yrs;med,IQR)	53.4 (44-63)	53.4 (44-60)	53.2 (44-62)	54.1 (47-60)	0.98
Female sex	20 (50%)	29 (55%)	28 (65%)	33 (76%)	0.006*
Duration (yrs) F	NA	2.4 (1.4-5.3)	5.0 (1.3-9.1)	8.1 (4.3-17.0)	0.001*
sBP (mmHg) F	144 (127-170)	139 (123-158)	139 (117-157)	145 (127-174)	0.15
dBp (mmHg) F	83.0 (73.5-95)	82 (75.5-91)	80 (73-96)	86 (78-92)	0.55
BMI >25 kg/m ²	19 (47.5)	33 (62.3)	28 (65.1)	25 (58.1)	0.34
HbA1c (IFCC) ‡	29.1 (4.9)	55.0 (25.5)	62.2 (28.7)	70.7 (23.5)	0.001*
HbA1c (NGSP%) ‡	4.8 (0.5)	7.2 (2.3)	7.8 (2.6)	8.6 (2.2)	0.002*
Hb (g/dl;mean;SD)	15.1 (2.1)	14.3 (2.0)	13.8 (2.1)	13.6 (1.6)	0.004*
Anaemia	6 (15%)	7 (13%)	7 (16%)	7 (16%)	0.76
HIV positive	2 (5%)	10 (19%)	6 (14%)	4 (9%)	0.82
Total chol >5mmol/L	8 (20%)	16 (30%)	15 (35%)	19 (44%)	0.017*
HDL (mmol/L) ‡	0.86 (0.37)	0.94 (0.31)	1.02 (0.38)	1.14 (0.38)	0.004*
LDL (mmol/L) ‡	2.16 (1.17)	2.31 (0.78)	2.47 (0.98)	2.80 (0.77)	0.005*
Triglycerides ‡	1.06 (0.82)	1.85 (1.81)	1.44 (0.98)	1.68 (0.97)	0.005*
↑ Urine ACR†	7 (18%)	13 (25%)	14 (33%)	23 (53%)	0.003*

≠ χ^2 test for trend, ANOVA, or Kruskal Wallis tests as appropriate. * Denotes statistical significance
‡ mean, SD; F median, IQR; † Raised urine ACR: male >2.5 mg/mmol; female >3.5)mg/mmol

10.4.2 Exploratory analysis

Levels of CRP, VEGF, VCAM, E-selectin and ICAM-1 (measured at recruitment to the MDRS) were weakly correlated with each other although the values were significantly different than zero (Table 10.2). The correlation coefficient between CRP and VEGF was 0.362 ($p=0.001$), between CRP and sVCAM-1 was 0.348 ($p=0.001$) and between CRP and E-selectin was 0.326 ($p=0.001$). RhPAT was strongly correlated with FRHI (correlation coefficient 0.713, $p=0.0001$) but not AI. All other factors were less strongly correlated (Table 10.2). E-selectin level correlated with HbA1c (correlation coefficient 0.713, $p=0.001$). AI correlated strongly with age (correlation coefficient 0.802, $p=0.001$).

Table 10.2 Correlation coefficients for serum markers of endothelial dysfunction and measures of clinical endothelial function in 179 subjects.

		CRP	VEGF	VCAM	E-selectin	ICAM	rhPAT	AI
Serum markers	CRP (mg/L)	•	•	•	•	•	•	•
	VEGF (pg/mL)	0.362	•	•	•	•	•	•
	VCAM (ng/mL)	0.348	0.203	•	•	•	•	•
	E-selectin (ng/mL)	0.326	0.065	0.058	•	•	•	•
	ICAM (ng/mL)	0.299	0.094	0.160	0.262	•	•	•
Measures of endothelial function	rhPAT	-0.034	0.069	-0.025	0.005	0.010	•	•
	AI	0.089	0.023	0.118	-0.048	0.062	0.189	•
	FRHI	-0.006	-0.032	-0.014	-0.019	0.046	0.713	0.106

10.4.3 Comparison between the 4 study groups

Levels of serum VEGF in subjects from each of the four study groups are shown in Figure 10.1. The fitted multiple linear regression model showed a significant difference in mean serum VEGF between subjects with and without diabetes but not between subjects with and without diabetic retinopathy or between subjects with and without STDR (Table 10.3). Higher serum level of E-selectin was detected in those with diabetes, although the result did not reach the statistical significant threshold ($p=0.066$).

The presence of DR and STDR was not significantly associated with serum levels of E-selectin ($p=0.43$ for DR; $p=0.33$ for STDR). The presence of diabetes, DR and STDR was not significantly associated with serum levels of VCAM-1 ($p=0.95$ for diabetes; $p=0.61$ for DR; $p=0.79$ for STDR), ICAM-1 ($p=0.053$; $p=0.44$; $p=0.48$), or CRP ($p=0.16$; $p=0.79$; $p=0.14$), or with PAT ($p=0.14$; $p=0.90$; $p=0.470$), AI ($p=0.061$; $p=0.85$; $p=0.40$) or FRHI ($p=0.33$; $p=0.84$; $p=0.40$). When considering subjects with HIV ($n=22$) and without HIV ($n=148$), those with HIV demonstrated higher serum level of E-selectin (unpaired t-test, $p=0.015$). Levels of CRP, VEGF, VCAM-1, ICAM-1, rhPAT, FRHI and AI were not significantly different between subjects with HIV and without HIV (unpaired t-test).

Figure 10.1 Baseline serum levels of vascular endothelial growth factor (VEGF) in subjects without diabetes ('Control', n=40), with diabetes but no diabetic retinopathy ('No DR', n=53), with diabetic retinopathy ('DR', n=43), and with sight threatening diabetic retinopathy ('STDR', n=43). Bars indicate mean and 95% CIs for the population means.

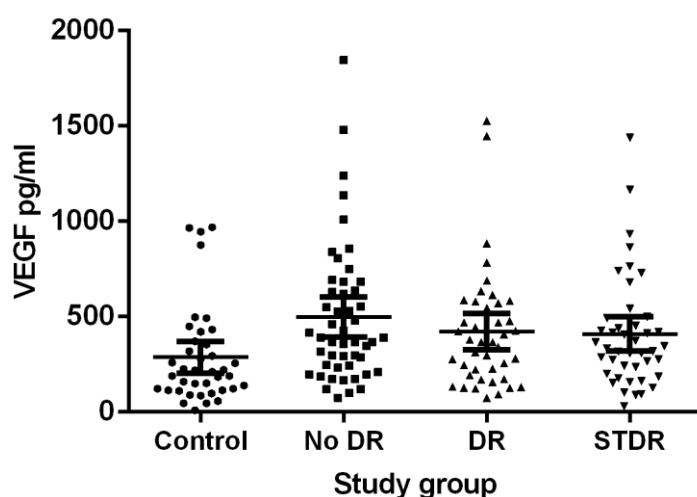


Table 10.3 Coefficients, 95% CIs and p-values of the multiple linear regression model to assess the association of serum VEGF level with presence of diabetes, diabetic retinopathy and sight threatening diabetic retinopathy (STDR) in 179 subjects.

Presence of	Parameter estimate	95% CI for parameter	p value
Diabetes (Y/N)	209.3	77.2 - 341.4	0.002*
Diabetic retinopathy (Y/N)	-77.0	-205.7 – 51.6	0.24
STDR (Y/N)	-11.8	-144.6 – 120.9	0.86
Intercept (Y/N)	288.0	189.5 – 386.6	0.0001

10.4.4 Logistic regression analysis

The multivariate regression model that was fitted in the previous section indicated that elevated serum VEGF is positively associated with having diabetes in this population (Table 10.3). Presence of diabetes and E-selectin were positively correlated although the result was just outside the significance boundary. In this

section I present an analysis of the data using an alternative approach. I used multiple logistic regression where presence of diabetes is the dependent variable and the 8 factors described in Table 10.4 are the predictor variables. Serum level of VEGF and E-selectin was significantly, positively associated with presence of diabetes in multiple logistic regression.

Table 10.4 Associations of the presence of diabetes in 179 subjects (univariate and multivariate logistic regression)

	OR	95% CI	p value
Univariate logistic regression			
CRP (mg/L)	1.05	0.98 - 1.12	0.18
VEGF (pg/mL)	1.002	1.000 - 1.004	0.009*
VCAM-1 (ng/mL)	0.99	0.99 - 1.00	0.58
E-selectin (ng/mL)	1.03	1.00 - 1.05	0.019*
ICAM-1 (ng/mL)	1.002	1.00 - 1.01	0.049*
rhPAT index	2.01	0.99 - 4.09	0.053
Sex (male)	0.54	0.27 - 1.11	0.094
Age (years)	1.003	0.97 - 1.03	0.85
Multiple logistic regression			
VEGF (pg/mL)	1.002	1.000 - 1.004	0.014*
E-selectin (ng/mL)	1.030	1.004 - 1.056	0.022*
rhPAT index	1.962	0.936 - 4.112	0.074
Sex (male)	0.564	0.252 - 1.264	0.17

10.5 Results: analysis of progression data

10.5.1 Participants

I studied endothelial function in relation to progression of DR at 24 months. Three groups of subjects (described in Section 10.3.1) were included in this analysis: subjects with diabetes but no DR at baseline (Group 2), those with DR but not STDR (Group 3) and those with STDR (Group 4). Progression of DR would not occur in those subjects without diabetes (Group 1) who were therefore excluded from this analysis. Characteristics of subjects are shown in Table 10.5. Two (or more) step progression (from baseline) was observed at 24 months in 24 subjects (19.2%; 12.3-26.1 95% CI).

Table 10.5 Characteristics of subjects included in the MDRS endothelial study progression analysis categorised by diabetic retinopathy status at baseline.

DR=diabetic retinopathy; STDR=sight threatening diabetic retinopathy.

	Group 2	Group 3	Group 4	Total
Baseline DR status	No DR	Dr no STDR	STDR	
Subjects	53	43	43	139
Subjects seen at 24 months	48 (91%)	40 (93%)	37 (86%)	125 (90%)
Confirmed dead at 24 months	3	3	4	10 (7%)
Subjects lost to follow-up	0	2	2	4 (3%)
2-step DR progression	4 (8.3%)	9 (22.5%)	11 (29.7%)	24 (19.2%)

10.5.2 Comparison between progression groups

Figure 10.2 shows baseline levels of endothelial biomarkers in subjects who demonstrated progression of retinopathy by 2 (or more) steps at 24 months (n=24) and those who did not (n=101). Neither VEGF, ICAM-1, E-selectin, VCAM-1, CRP or rhPAT index at baseline were associated with progression of DR by 2 (or more) steps on the LDES scale at 24 months based on univariate or multivariate regression analyses (Table 10.6).

Figure 10.2 Baseline serum levels of (A) vascular endothelial growth factor (VEGF), (B) E-selectin, (C) intercellular adhesion molecule-1 (ICAM-1) and (D) rhPAT index in subjects who demonstrated 2 (or more) step progression of diabetic retinopathy on the Liverpool Diabetic Eye Study scale at 24 months (n=24) and those who did not (n=101). Bars indicate mean and 95% CIs for the population means.

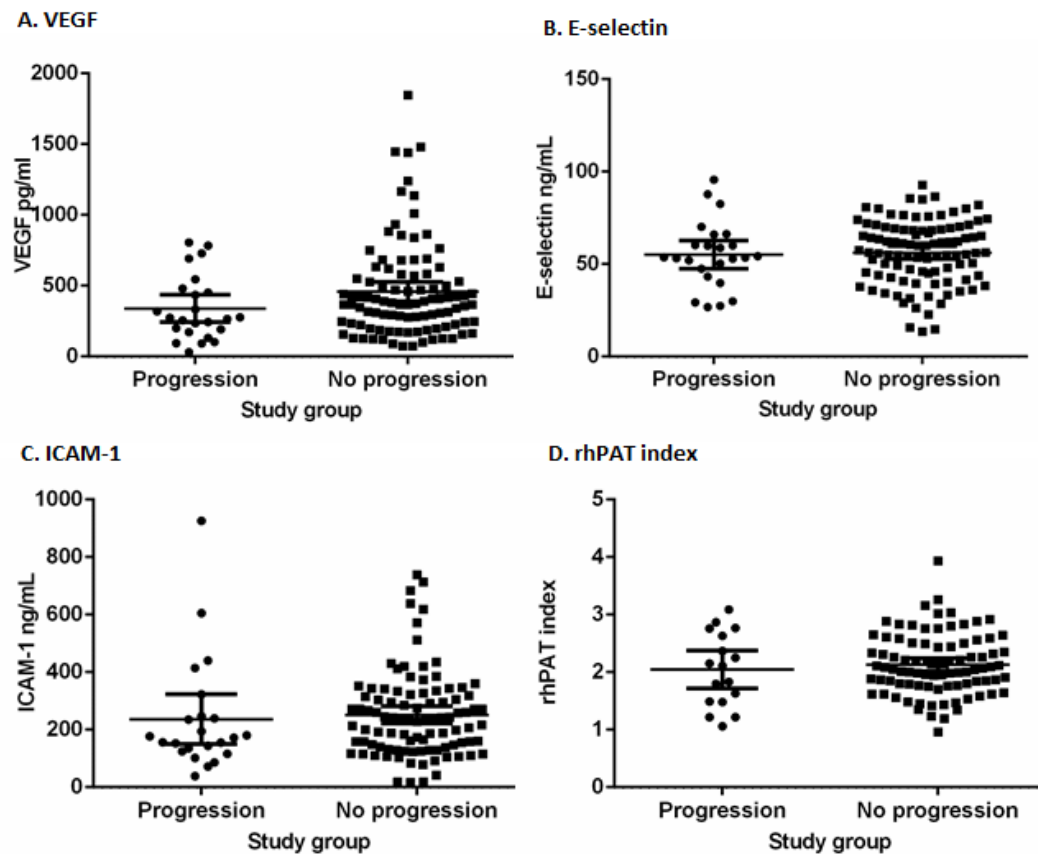


Table 10.6 Risk factors for association of progression of retinopathy by 2 (or more) steps on the LDES scale at 24 months in 135 subjects.

	OR	95% CI	p value
Univariate logistic regression			
CRP (mg/L)	0.99	0.97 - 1.03	0.90
VEGF (pg/mL)	0.99	0.99 - 1.00	0.11
VCAM-1 (ng/mL)	1.00	0.99 - 1.00	0.54
E-selectin (ng/mL)	0.99	0.97 - 1.02	0.78
ICAM-1 (ng/mL)	0.99	0.99 - 1.00	0.68
rhPAT index	0.74	0.28 - 1.97	0.54
Sex (male)	0.32	0.10 - 0.99	0.05
Age (years)	1.01	0.97 - 1.04	0.80
Multivariate logistic regression			
VEGF (pg/mL)	0.99	0.99 - 1.00	0.15
Sex (male)	0.33	0.09 - 1.25	0.10

10.5.3 Comparison between subjects who died and those who survived

I compared levels of serum markers of endothelial dysfunction and the rhPAT index measured at baseline in subjects who died (n=10) and those who were alive at 24 months (n=125). Figure 10.4 shows baseline levels of VCAM-1 and E-selectin in these 2 groups. Serum VCAM-1 but not E-selectin was associated with death in multivariate regression (Table 10.7).

Figure 10.4 Baseline serum levels of (A) vascular cell adhesion molecule 1 (VCAM-1) and (B) E-selectin in subjects who died (n=10) and those alive (n=125) at 24 months in the Malawi Diabetic Retinopathy Study. Bars indicate mean and 95% CIs for the population means.

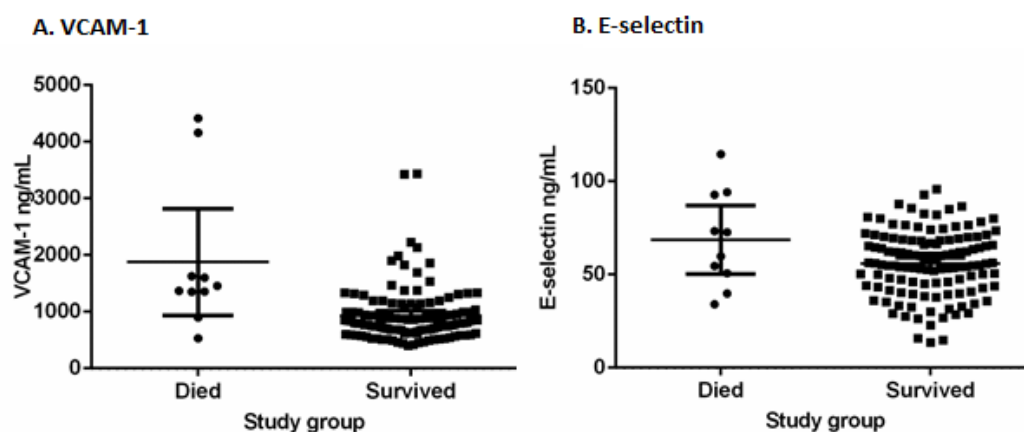


Table 10.7 Risk factors for association of death in 135 subjects over 24 months in the Malawi Diabetic Retinopathy Study (univariate and multivariate logistic regression). Endothelial biomarker measurements taken at baseline.

	OR	95% CI	p value
Univariate logistic regression			
CRP (mg/L)	1.02	1.00-1.04	0.025*
VEGF (pg/mL)	1.00	0.99-1.00	0.18
VCAM-1 (ng/mL)	1.00	1.00-1.01	0.001*
E-selectin (ng/mL)	1.04	1.00-1.08	0.040*
ICAM-1 (ng/mL)	1.00	0.99-1.01	0.18
rhPAT index	0.75	0.20-2.75	0.66
Sex (male)	1.27	0.34-4.75	0.72
Age (years)	1.05	0.99-1.11	0.10
Multivariate logistic regression			
VCAM-1 (ng/mL)	1.01	1.00-1.01	0.011*
E-selectin (ng/mL)	1.05	0.99-1.11	0.098
Age (years)	1.06	0.97-1.16	0.18

10.6 Discussion

10.6.1 Principal findings

At baseline, levels of CRP, VEGF, VCAM, E-selectin and ICAM-1 were weakly correlated with each other. These biomarkers were studied because of previous work demonstrating associations with microangiopathy in diabetes [46-54]. Subjects with diabetes demonstrated higher levels of serum VEGF. Higher serum VEGF and E-selectin were associated with having diabetes in multivariate regression. E-selectin level correlated with HbA1c and was higher in subjects with HIV. In analysis of progression data there was no significant difference between those subjects who demonstrated 2 (or more) step progression at 24 months and those who did not in terms of baseline level of CRP, VEGF, VCAM-1, E-selectin, ICAM-1 and rhPAT index. Subjects who died demonstrated higher baseline levels of CRP, VCAM-1 and E-selectin. Serum VCAM-1 was associated with death in multivariate regression.

10.6.2 Comparison with studies of endothelial function in diabetes

The results of this study demonstrate the first evidence of endothelial dysfunction in Malawian subjects with diabetes. A large body of evidence indicates that the pathophysiological mechanisms involved in the development of diabetes and its vascular complications include chronic low grade inflammation, endothelial dysfunction and pro-coagulant imbalance. An excellent review of this topic has been produced by Goldberg [343]. Serum levels of soluble adhesion molecules (namely ICAM-1 and E-selectin) were independent predictors of development of diabetes in the Nurses Health cohort study [344]. Elevated s-ICAM1 has been previously demonstrated in type 1 [345] and type 2 [346] diabetes.

Elevated VEGF has been shown previously in subjects with diabetes compared to control subjects without diabetes [347-350] including one study from North Africa [351]. A potential confounder to this relationship has been suggested by Schlingemann *et al* [352]. These authors suggest that artificial *ex-vivo* release of VEGF from platelets in both serum and plasma samples, which correlates with

glycaemic control, may be responsible for the differences reported between subjects with and without diabetes. Exploring this relationship in Malawian subjects was beyond the scope of the MDRS.

10.6.3 Comparison with studies of endothelial function and microvasculopathy in diabetes

The MDRS did not show a difference in markers of endothelial dysfunction between subjects with varying grades of retinopathy. Nor did my work show differences in these markers between subjects in whom retinopathy progressed and those in whom it did not. In contrast there is a considerable body of evidence to suggest that inflammatory processes and endothelial dysfunction are key elements in the pathogenesis of microvasculopathy in diabetes [343]. Inflammatory and endothelial biomarkers are associated with and predictive of microangiopathy. Cross-sectional studies in both type 1 and type 2 diabetes have shown elevated levels of biomarkers including CRP, von Willebrand factor (vWF) and E-selectin in subjects with nephropathy [353,354]. Baseline E-selectin and fibrinogen levels were predictive of development of nephropathy in the Diabetes Control and Complications Trial (DCCT) cohort (type 1 diabetes) [355].

Consistent with studies on nephropathy, biomarkers of endothelial dysfunction are associated with presence and incidence of DR [343]. Elevated soluble ICAM-1, VCAM-1 and E-selectin have been demonstrated in DR in type 2 diabetes [49]. In the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) soluble VCAM, tumour necrosis factor- α (TNF- α) and homocysteine levels were associated with prevalence of more severe grades of DR in subjects with nephropathy; these markers were not associated with progression of DR at 15 years [356]. In the Dutch 'Hoorn' population-based, cohort study of diabetes composite scores of markers of both inflammation (CRP and ICAM-1) and endothelial dysfunction (vWF, VCAM-1 and uACR) were associated with presence of DR [48]. In a cohort study of 725 African Americans with type 1 diabetes plasma levels of E-selectin were associated with progression of DR, E-selectin and TNF- α levels with progression to PDR and

sICAM-1 and TNF- α levels with incidence of macular oedema [357]. These observations are consistent with the hypothesis, supported by work in mice, that retinopathy is associated with low grade chronic inflammation [358]. It is suggested that VEGF induces up-regulation of adhesion molecules, including ICAM-1 and VCAM-1, facilitating leucocyte adhesion and stasis in the retinal vasculature and ultimately endothelial perturbation and cell death [359].

10.6.4 Comparison with studies of endothelial function and mortality in diabetes

I have demonstrated an association between markers of endothelial function and mortality in diabetes. Subjects who died showed higher baseline levels of VCAM-1, E-selectin and CRP. Serum VCAM-1 was associated with death in multivariate regression. The MDRS is the first study to demonstrate this relationship in African subjects. E-selectin level correlated with HbA1c and was higher in subjects with HIV. It was not possible to accurately assess cause of death for subjects who died during the MDRS. However, diabetic micro- and macro-vasculopathy are likely to be strong contributors.

The Dutch 'Hoorn' population-based study showed an association between levels of vWF, sVCAM-1, sICAM-1 and CRP and cardiovascular mortality in type 2 diabetes [46,47]. In a Danish 9 year prospective study of type 2 diabetes sVCAM-1 and CRP were associated with mortality [354]. Taken together, the results of these reports and the present study are consistent with a role for endothelial dysfunction in the fatal micro and macrovascular complications of diabetes. Associations with individual markers are not consistent. In part this may be due to large biological variation in expression and measurement of these biomarkers. These studies support a role for low-grade chronic inflammation, the expression and up-regulation of adhesion molecules and leucocyte adhesion and stasis in the vascular complications of diabetes.

10.6.5 Microvascular reactivity in diabetes

The MDRS did not show evidence of decreased endothelial function, as assessed by peripheral artery tonometry, either in analysis of baseline or progression data. Contrary to what would be expected in endothelial dysfunction, subjects with diabetes demonstrated higher rhPAT index although the relationship was not statistically significant. rhPAT index was not associated with varying degrees of DR. Neither rhPAT index, augmentation index or FRHI were associated with DR progression or mortality. Lower rhPAT index has been demonstrated in children [66] and adolescents [360] with type 1 diabetes and in adults with type 2 diabetes [361] in the developed world. Recently Lim et al [362] showed an association between increasing severity of DR and increasing mean RHI and mean AI (measured with the EndoPAT device) in 95 Chinese subjects. The authors used multivariate analysis adjusting for age, gender, duration of diabetes, smoking, HbA1c and hypertension. Flow mediated dilatation (FMD) is an alternative non-invasive test of endothelial function (discussed in Chapter 1, Section 1.5.5). Lower FMD has been demonstrated in subjects with diabetes compared to control subjects without diabetes [363] and in subjects with DR when compared to those with diabetes but no retinopathy [364,365].

Explanations for a lack of difference in endothelial function as assessed by PAT in this study include poor performance of the test. The EndoPAT has been shown to provide an acceptable level of reproducibility [366] and should not be operator dependent. Reassuringly the range of values reported above are within the expected range of the test. In my study no positive control was used. The EndoPAT test has been validated for the diagnosis of coronary artery disease against the reference standard of acetyl choline angiography [65]. Facilities for assessing coronary artery disease are extremely limited in Malawi and assessment of the EndoPAT test in this way was not possible. The influence of environmental factors, either short acting (e.g. dietary components) or long term (e.g. domestic smoke exposure), on endothelial function and possible physiological differences between the Malawian and previously tested populations with regard to the test cannot be

ruled out. Diabetic neuropathy may lead to vasodilation. This is unlikely to have exerted a significant influence on our findings.

10.6.6 Limitations of this work

The pathophysiology of DR is multifaceted. Inflammation and endothelial dysfunction are complex entities that cannot be accurately reflected by a small number of biomarkers. It is difficult to measure the endothelium in isolation and multiple influences including pharmaceutical agents, as well as diabetes, are likely to affect its function. This may account for the weak internal correlation between markers of endothelial dysfunction in the MDRS. Individual markers will be affected by particular health states. For example CRP level is strongly affected by intercurrent infection and other inflammatory conditions [367]: an important confounder. The retinal endothelium is a small component of the microvascular endothelium and microvascular events may be masked by activation of the macrovascular endothelium. The retinal vasculature is distinct from other components of the vascular system [368]; markers measured in peripheral blood may be poorly representative of retinal endothelial function.

Endothelial dysfunction has been demonstrated in many acute conditions including sepsis [369] and malaria [370] where profound vascular disturbance occurs.

Diabetes is a chronic disease in which a baseline of endothelial function is difficult to establish. Endothelial dysfunction is evident prior to clinical manifestations of microvascular complications [344]. However, endothelial health may vary over time (e.g. with varied glycaemic control [371]) and single measurements of biomarkers may therefore present an incomplete picture of vascular health. Future work on endothelial function in Malawian subjects with diabetes could include investigation of other endothelial biomarkers including angiopoietins, analysis of the activation status of circulating leucocyte sub-classes by Fluorescence-Activated Cell Sorting (FACS) and direct endothelial cell histology and/or culture following sub-cutaneous fat biopsy.

10.7 Chapter summary

The results presented in this chapter provide the first evidence from sub-Saharan Africa of endothelial dysfunction in subjects with diabetes and of an association between levels of endothelial biomarkers and mortality in these subjects.

Endothelial biomarkers are unlikely to be useful for diagnosis of diabetes in a low resource setting and my data do not support the use of these biomarkers to identify patients at high risk of DR. However, inflammatory activity and endothelial dysfunction are potentially important contributors to the macro- and micro-vascular complications of diabetes. These data add to the literature on endothelial dysfunction in diabetes but do not provide a basis for testing the effects of treatments aimed at decreasing inflammatory activity and improving endothelial function as a means of preventing or limiting morbidity and mortality. Further work to study the role of the endothelium in the vascular complications of diabetes in sub-Saharan Africa may be justified. However, it may be challenging to produce informative results.

Chapter 11. Discussion

11.1 Aims of the chapter

In this chapter I summarise the findings of this thesis and compare these results to previously published work in the same field. I will detail the strengths and weakness of the work I have performed and then go on to discuss the epidemiological, pathophysiological and health policy implications of my results. Finally I will describe ongoing work that has arisen from these results and provide suggestions for future work.

11.2 Summary of findings

At the outset of this work I hypothesised that, in the Malawian population, diabetic retinopathy (DR) is more common and progresses more quickly than that seen in developed countries (Chapter 4, Section 4.7.3). I suggested that factors particular to this population might alter the spectrum of disease compared to that observed in the West, and that these factors include anaemia, co-infection with HIV and poorly controlled blood pressure. I hypothesised that endothelial dysfunction is an important pathophysiological mechanism in DR; endothelial perturbation results in exhaustion of the mechanisms for endothelial self-repair leading to the development of maculopathy and ischaemic retinopathy.

In order to test these hypotheses I recruited a cohort of subjects with diabetes from Southern Malawi. Subjects were systematically sampled from two hospital-based, primary care diabetes clinics. Visual acuity, glycaemic control, systolic blood pressure, HIV status, urine albumin–creatinine ratio, and haemoglobin and serum lipid levels were assessed. Retinopathy was graded at an accredited reading centre using modified Wisconsin grading of four-field mydriatic photographs. Subjects were recalled for assessment at 12 and 24 months. Subjects who did not attend for follow-up visits were systematically traced. Additionally, a previously studied cohort of subjects with diabetes was traced and recalled for assessment 5 years

after their recruitment to a cross sectional study of the complications of diabetes at Queen Elizabeth Central Hospital (QECH), Blantyre in 2007. Endothelial function was explored in a nested case-control study in the main MDRS cohort. Serum markers of endothelial dysfunction and peripheral artery tonometry (PAT; an *in vivo* measure of peripheral vascular endothelial health) were compared across four groups: subjects without diabetes, subjects with diabetes and DR, subjects with DR but not sight threatening diabetic retinopathy (STDR) and subjects with STDR.

The Malawi Diabetic Retinopathy Study (MDRS) is the first prospective cohort study of DR from Sub-Saharan Africa (SSA). Features which differentiate this work from previous cross sectional studies include the high prevalence of infective disease (malaria and HIV) and anaemia in the cohort, robust external validation of retinopathy grading at an accredited reading centre, data on the number of patients requiring laser treatment (new prevalent cases) and a comprehensive assessment of systemic parameters including HbA1c, urine ACR and haemoglobin level.

Prevalence of retinopathy 357 patients were recruited to the MDRS. At baseline 13.4% subjects were HIV positive; 15.1% were anaemic. The overall prevalence of any retinopathy, STDR and proliferative DR (PDR) was 50.1% (95% CI 44.9–55.3), 29.4% (24.7–34.1) and 7.3% (4.6–10.0), respectively. In multivariate logistic analysis the presence of STDR was associated with duration of diabetes (years) (OR 1.11, 95% CI 1.05-1.17), HbA1c (%) (OR 1.31, 1.13-1.50), sBP (mmHg) (OR 1.03, 1.01-1.04), haemoglobin (g/dl) (OR 0.80, 0.68-0.95) and LDL cholesterol (mmol/L) (OR 1.63, 1.18-2.25). No significant association with HIV status was demonstrated.

Incidence and progression of retinopathy Of 357 subjects recruited to the main MDRS cohort, 295 were assessed at 24 months, 28 were confirmed dead, 20 declined assessment and 14 were not traced (90.5% follow-up). At follow-up (median 2.0yrs), in subjects with no retinopathy at baseline, cumulative incidence of any retinopathy was 38.0% (95% CI 30.2-45.8). 24 month rates of progression were: 2 step (or greater) 58/293 (19.8%); STDR 23/225 (10.2%). Cumulative incidence at 24 months of STDR for subjects with Level 10 (no retinopathy), Level 20

(background) and Level 30 DR at baseline were 2.7% (95% CI 0.1-5.3), 27.3% (16.4-38.2) and 25.0% (0-67.4), respectively. In multivariate logistic analysis 2 step progression of DR was associated with HbA1c (OR 1.27, 95%CI 1.12-1.45), baseline grade of DR (1.39, 1.02-1.91) and HIV infection (OR 0.16, 0.03-0.78).

Of those subjects recruited in 2007 a total of 135 (48% of the original cohort) were assessed. At median follow-up of 5.3 years in subjects with no retinopathy at baseline incidence of any DR was 48.4% (38.2-58.5). 5 year rates of progression were: 2 step (or greater) 48/132 (36.4%; 95% CI 28.2-44.6); 3 step (or greater) 30/132 subjects (22.7%; 15.6-29.9); STDR 34/115 (29.6%; 21.3-37.9). 5 year incidence of STDR for subjects with Level 10, and Level 20 DR at baseline were 19.4% (11.3-27.4) and 81.3% (62.1-100), respectively. In multivariate logistic analysis 2 step progression of DR was associated with higher mean HbA1c (OR 1.47, 95%CI 1.13-1.90), longer duration of diabetes (1.08, 1.00-1.17) and lower haemoglobin (OR 0.80, 0.63-0.99).

Vision and laser treatment At baseline 2.2% and 1.4% of subjects in the MDRS cohort had visual acuity (VA) worse than 60 letters (equivalent to 6/18 Snellen) and less than 50 letters (6/60 Snellen) in the better eye, respectively. Of patients with VA less than 80 letters (6/12 Snellen) DR was the sole or equal contributing cause in 49% of cases. During the course of the study 85 people required laser photocoagulation (63 scatter treatment with or without macular laser, 22 macular laser alone). In this treated cohort, rates of progression to visual loss were: ≥ 15 letters lost 17 subjects (5.8%), moderate visual impairment (<60 letters) 3 (1.0%), severe visual impairment (<50 letters) 5 (1.7%).

Mortality in the MDRS 28 subjects died during the MDRS. Cumulative incidence of death in the whole MDRS cohort at 12 and 24 months was 4.3% (2.2-6.4 95% CI) and 8.0% (5.1-10.9), respectively. Cumulative incidence of death amongst subjects with STDR at baseline at 12 and 24 months was 7.6% (2.6-12.6) and 13.2% (6.8-19.6), respectively. Cumulative incidence of death amongst HIV positive subjects at 12 and 24 months was 10% (1.7-18.3) and 18.1% (7.4-28.8), respectively. In

univariate analysis death was associated with STDR (OR 2.51; 95% CI 1.15-5.48; $p=0.021$), PDR (OR 6.47; 2.51-16.7; $p=0.0001$), HIV (OR 3.72; 1.54-9.00; $p=0.003$) and moderate visual impairment (OR 8.21; 2.48-27.1; $p=0.001$).

Endothelial function 179 subjects were included in the case control study of endothelial function: 139 from the MDRS plus 40 subjects without diabetes. At baseline, higher serum VEGF and E-selectin were associated with having diabetes in multivariate regression. The presence of diabetes, DR and STDR was not significantly associated with serum levels of VCAM-1, ICAM-1, or CRP or with PAT score. E-selectin level correlated with HbA1c and was higher in subjects with HIV. Analysis of progression data showed no significant difference between those subjects who demonstrated 2 (or more) step progression at 24 months and those who did not in terms of baseline level of CRP, VEGF, VCAM-1, E-selectin, ICAM-1 and PAT score. Subjects who died demonstrated higher baseline levels of CRP, VCAM-1 and E-selectin. Serum VCAM-1 was associated with death in multivariate regression.

11.3 Comparison with published data: prevalence and incidence of DR, vision and mortality

Prevalence of retinopathy Baseline data from the MDRS cohort is compared with previous data from Africa in Chapter 6, Section 6.5.2, and with studies from outside Africa in Section 6.5.3. Prevalence of any DR, PDR and maculopathy in the MDRS was higher than population based studies from Egypt [239], Mauritius [212,215] and Kenya [A. Bastawrous personal communication] and comparable with clinic based studies in the last decade from South Africa [224,241]. The prevalence of STDR in the MDRS was approximately 4 times that reported in recent European studies; the prevalence of PDR approximately 10 times higher.

Incidence and progression of retinopathy The MDRS is the first high-quality, prospective, cohort study of DR from SSA. Chapter 8, Section 8.5.6 compares data from the MDRS to cohort studies from Europe and North America. Progression to

STDR at 24 months from no retinopathy and background DR occurred approximately 3 and 2.5 times more frequently than reported in recent European studies, respectively. MDRS 5 year progression data is compared with studies from Africa and then Europe and North American in Chapter 9, Sections 9.5.4 and 9.5.5, respectively. 5 year progression to STDR from level 10 and level 20 DR was approximately 5 times and 3 times higher than that reported in recent European studies, respectively. 5 year progression to PDR was similar to data from recent data from England [331] and a high quality study from Mauritius in the 1990s [212,215].

Vision Few cross sectional studies have investigated VA in people with diabetes in SSA. Baseline data from the MDRS cohort is compared with previous data in Chapter 6, Section 6.5.5. Prevalence of visual impairment in MDRS was lower than that recorded in the Diabetes in Egypt project [239] (results were reported for right eyes only as opposed to VA in the better eye) but similar to data from the Wisconsin Epidemiological Study of DR (WESDR) [328] and a recent study from Iceland [290]. To my knowledge no studies from Africa have reported longitudinal visual acuity (VA) data in subjects with diabetes. Incidence of visual impairment in the MDRS is compared to previous data in Chapter 8, Section 8.5.8 and Chapter 9, Section 9.5.6. It is important to note that subjects in the MDRS were part of a relatively well funded observational study which achieved good levels of follow-up, and had access to laser therapy and also treatment for other ocular pathology including cataract. Levels of visual loss in the MDRS are likely to be lower than in Malawian patients not enrolled in research studies. 24 month cumulative incidence of 'moderate' and 'severe' visual loss in the MDRS was higher than similar thresholds of visual impairment reported in the WESDR at 4 years. 5 year incidence of 'severe' visual loss in the MDRS 2007 cohort was higher than that reported in the WESDR at 4 years and also higher than that reported in the Beaver Dam Eye Study (not confined to subjects with diabetes) at 5 years [342].

Mortality Reporting mortality in subjects with diabetes was not a stated aim of the MDRS. Nonetheless this data is an interesting outcome from my work. 24 month

cumulative incidence of death in the whole MDRS cohort was 8.0% (5.1-10.9). 24 months cumulative incidence of death amongst subjects with STDR at baseline at was 13.2% (6.8-19.6); amongst HIV positive subjects it was 18.1% (7.4-28.8). A number of high quality studies have reported mortality in subjects with diabetes in Africa. Mortality rates from the population-based Mauritius cohort study of diabetes complications have been published pooled with studies from Fiji and Nauru. After median follow-up of 5.0 years mortality in subjects with known diabetes was 122/278 (43.9%) for men and 97/317 (30.6%) for women [372].

In a prospective study from Tanzania McLarty et al [373] reported 5 year mortality of 40.5% in those with insulin dependent diabetes mellitus (IDDM), 19.0% in subjects with non-insulin dependent diabetes mellitus (NIDDM) and 57.0% in subjects with indeterminate type. 50% of deaths in IDDM subjects were attributed to ketoacidosis; 24% of NIDDM deaths were due to cardiovascular and renal disease; 48% of deaths in indeterminate type diabetes were attributed to infection. Mortality rates in European and American studies are considerably less. The United Kingdom Prospective Diabetes Study (UKPDS) [132] reported all cause mortality across all study participants at mean 10.0 years follow-up to be 17.9% (16.7-19.0). In the more recent ACCORD study [138] at mean 3.5 years follow-up all cause mortality was 4.5% (4.1-4.9). Both these large trials studied subjects with type 2 diabetes. Crude mortality rates may be misleading; the study of age-standardised, cause-specific mortality in the MDRS would be helpful but is beyond the scope of this thesis.

11.4 Possible explanations for high prevalence and progression rates

Differences between prevalence, incidence and progression figures from the MDRS and recent European and North American work are likely to reflect multiple disparities between the populations studied. These include ethnicity (genetic factors), poor access to health services, late diagnosis of diabetes, inadequate drug supplies (sub-optimal primary prevention of complications) and presence of comorbidities including poorly controlled hypertension and infective disease.

Comparisons between studies must be made with caution in view of different study designs and different follow-up rates. Of particular note, mortality rates are likely to differ greatly between populations and are an important cause of data censoring. The effects of individual variables on the severity and progression of DR will be explored in the latter part of this chapter. Specifically, the role of the population specific risk factors anaemia and HIV will be discussed. Firstly I will explore the implications of high rates of prevalence and progression of DR for health policy makers and for clinical practice.

11.5 Implications for health policy makers

The costs of diabetes to Africa are significant, and are rising rapidly. Kirigia et al. [374] estimated that the total economic cost (direct and indirect) of diabetes in the WHO Africa region in 2000 was US\$67 billion: equivalent to US\$8,836 per person with diabetes per year. A significant proportion of this figure is accounted for by the opportunity cost of productive time lost due to permanent disability and premature mortality. The burden of diabetes and its complications is borne predominantly by the working age population [375]. DR is the commonest cause of blindness in the working population in the USA and Europe [376]. A disease which reduces the economic activity of this group affects individual, household and national economies.

The agenda for diabetes care in SSA is dominated at a national level by poorly resourced health services and at a community level by poverty. The International Diabetes Federation has estimated that in 2010 national funding for the care of diabetes in Africa was just US\$111 per person [377]. This figure is equivalent, on average, to 7% of national healthcare expenditure but varies widely between countries. In Malawi the total annual per capita expenditure on all healthcare was only US\$77 [16]. Opportunity costs will also be lower with the Gross National Income per capita only \$330, but it is clear that current expenditure is a fraction of the cost of the disease. Limited public funding means individual patients and their families are forced to spend significant proportions of their income on diabetes treatment. Diabetes care must compete with infective diseases and other

healthcare initiatives in terms of political and financial priorities. It does not lend itself to the vertical programs favoured by donors. However global political attention is now turning to non-communicable diseases (NCDs), as witnessed by the United Nations high-level meeting on NCD prevention and control, September 2011 [378]. Policy makers require evidence-based guidance on resource allocation.

In Chapter 4 of this thesis I highlighted the lack of epidemiological data on the complications of diabetes in SSA. Specifically, high-quality cohort studies and population-based cross-sectional studies are urgently required by policy makers planning the introduction of diabetes services in the region. The MDRS is the first prospective, cohort study of DR from SSA. Results from this work: prevalence, incidence and progression of DR in a mixed rural and urban population attending diabetes clinics in one country in SSA represent critical baseline data for future studies. It is against data such as this that future interventions will be judged.

11.6 Implications for clinical practice: barriers to effective care delivery

Data from the MDRS highlight the urgent need for provision of services for retinopathy detection and management to avoid a large burden of vision loss. Provision of effective care for diabetes and its complications requires not only resource allocation but reorganisation of health systems. Services in SSA have traditionally been organised to manage distinct health events, principally episodes of infectious diseases, maternal and perinatal disorders and trauma. NCDs such as diabetes demand effective, integrated multidisciplinary services over a lifetime. This is a significant challenge for health providers as it impacts on all elements of health systems: workforce, facilities, technology and pharmaceuticals as well as leadership and governance. Reductions in microvascular complications from improved glycaemic and blood pressure control, as shown in the Diabetes Complications and Control Trial (DCCT) [12] and the UKPDS [14], will be maximised if monitoring and medications are consistently available. Similarly, provision of services for detection

and management of complications, including DR, must be consistent rather than intermittent.

Diabetes can be thought of as an index case for NCD healthcare delivery in Africa and developing countries worldwide. The World Health Organisation (WHO) has identified the following problems for healthcare delivery in developing countries: lack of organisational structure for chronic disease care; minimal staffing and training provided to healthcare workers; minimal communication with the public to address preventative strategies; non-existence of organised healthcare information systems; and lack of involvement and integration with other community resources [379]. In Africa, these barriers translate into the inadequacies in diabetes care identified by Whiting et al. [380] listed in Box 11.1. In a recent article in the journal BMC Medicine I, together with colleagues from Liverpool and Malawi, identified a number of specific barriers to DR care in Africa listed in Box 11.2 [381].

Box 11.1 Inadequacies in diabetes care in Africa as identified by Whiting et al. [380]

1. Poor patient attendance at clinics
2. Low doctor to patient ratio leading to short consultation times and little or no time for patient education
3. Low staff levels including a lack of trained nurses and other health workers
4. Lack of staff training specific to diabetes
5. A lack of systematic evaluation and monitoring of the complications of diabetes
6. Non-existent or inadequate referral systems
7. Poor record keeping
8. Non-existent diabetes multidisciplinary healthcare teams
9. Lack of infrastructure to support services
10. Lack of national policies

Box 11.2 Specific barriers to diabetic retinopathy care in the African region identified by Burgess et al [381].

1. Lack of ophthalmologists
2. Low number of ophthalmologists with training and experience in management of DR
3. Low numbers of opticians and ophthalmic clinical officers (OCOs) to perform opportunistic screening; commercial opticians are only accessible to the wealthy
4. Lack of training for opticians and OCOs in fundoscopy
5. Inadequate referral systems from primary to secondary care and from medical departments to ophthalmic services
6. Non-existent systematic screening programs
7. Little access to imaging technology including fluorescein angiography and optical coherence tomography
8. Lack of treatment infrastructure including lasers and laser maintenance

The evolving epidemic of diabetes in Africa necessitates a coordinated response that involves integrating services at a number of levels. In the community, interventions for the prevention and control of NCDs are necessary. Several models exist in South Africa, for example the Community Health Intervention Programme, the efficacy of which is currently being evaluated [382]. Primary prevention of complications by systemic risk factor management is a priority. This requires the expansion of health centres and also hospital-based diabetes clinics. With a rapid increase in patient numbers, a simplification of some services has been proposed. This might resemble the streamlining of HIV services that was necessary to achieve antiretroviral therapy roll out in many states in SSA [383]. Moving away from individualised care in vascular risk management has significant drawbacks [384]. The DART study on routine versus clinically driven monitoring of ART for HIV showed that early on a high volume, low complexity approach is non-inferior to individualised care [385]. But later, as the disease becomes more complex, individualised care is superior [386]. The same may not be true for diabetes where

many patients present late and the seeds of complications are sown from the outset.

Development of specialist services to detect and manage complications is required supported by robust referral mechanisms. The effectiveness of laser photocoagulation in reducing the likelihood of visual impairment and blindness in patients with PDR [18] and macular oedema [19] is well established. Recent evidence demonstrates better outcomes in the short to medium term from intra-vitreous anti-VEGF agents (injected into the vitreous) in diabetic maculopathy that has already affected vision [387]. This topic is the subject of a recent Cochrane review [388]. At present these agents which require multiple repeat injections, are prohibitively expensive for widespread use in resource-poor countries (approximately US\$800 per injection for the drug alone). However, off-label use of the anti-VEGF agent bevacizumab (approximately US\$70 per injection), is used in some African centres on a paying patient basis, an approach supported by the BOLT study [389]. Vitreoretinal surgery has an important role in managing advanced disease. Unfortunately published data from this setting is sparse and more research on long-term outcomes and cost effectiveness is required.

Provision of laser services requires substantial initial investment in equipment and training of ophthalmologists. However, equipment upkeep costs are small and there are no on-going drug costs. The inadequacy of retinal training and paucity of referral networks are significant barriers to service development for DR. I, together with colleagues from Liverpool and Malawi, have produced a number of proposals to confront these issues listed below in Box 11.3. Our clinical and research group have demonstrated that provision of a laser treatment service is feasible in Blantyre, Malawi albeit with external support. In Queen Elizabeth Central Hospital, set-up costs of equipment and training of ophthalmologists has been funded by an outside agency: the World Diabetes Foundation. As part of the capacity building agenda of my PhD fellowship I have been involved in training ophthalmic clinical officers (OCO's) in the recognition and referral of DR and ophthalmology registrars in performing retinal laser.

Box 11.3 Proposals by Burgess et al [381] to improve retinal training and retinal referral networks in sub-Saharan Africa

1. Increase the number of ophthalmologists trained and working in the region to allow increased sub-specialisation
2. Provision of imaging and treatment infrastructure to allow sub-specialty practice
3. Creation of regional centres of excellence in Africa for provision of tertiary retinal care and training
4. Development of retinal research networks: providing funding both for personnel and equipment, facilitating income generation for eye units, setting standards for clinical practice, improving the evidence base for this setting, setting the political agenda and attracting excellent clinicians.
5. Prioritisation of sub-specialty development in post-graduate training programs
6. Promotion of partnership arrangements with retinal centres in developed countries to facilitate knowledge and skill sharing
7. Provision of retinal fellowships tailored to developing world trainees in retinal centres in developed countries
8. Use of donor and government funds to minimise costs of such fellowships for trainees on condition of return to practice in country of origin

11.7 Determinants of severity and progression: overview

In Chapter 6 I reported associations between prevalence of STDR in the main MDRS cohort and longer duration of diabetes, higher systolic blood pressure, higher HbA1c, higher LDL, and lower haemoglobin. The latter finding is novel and is discussed separately in Section 11.8. Progression of DR at 24 months was associated with higher mean HbA1c and higher baseline grade of retinopathy. Progression was negatively associated with HIV infection (discussed separately in Section 11.9). In the 2007 cohort (Chapter 9) 2 step progression at 5 years was associated with higher mean HbA1c, longer duration of diabetes and lower haemoglobin.

The association between glycaemic control and DR progression has been demonstrated previously in both observational (although not, to my knowledge, in a population in SSA) and intervention studies. Literature on this association is reviewed in Chapter 3, Section 3.5. In the Finnish Diabetic Nephropathy (FinnDiane) cohort study [390] of subjects with type 1 diabetes, progression of DR to a level requiring laser was associated with variability in glycaemic control (HbA1c variability when adjusted for mean HbA1c) [390]. While the limited number of assessments in the MDRS (baseline, 12 and 24 months) did not permit such an analysis, previous work at the QECH diabetes clinic shows highly variable glycaemic control in individual subjects (Ingrid Peterson, personal communication). This is to be expected due to intermittent drug supplies and may contribute to the high rates of DR progression observed in the MDRS.

In Chapter 3, Section 3.5.1 I discussed the potential disadvantages of HbA1c when compared to measurement of fasting plasma glucose (FPG): difficulties in quality control and test standardisation as well as variability with haemoglobin variants, anaemia and HIV infection. In Chapter 5, Section 5.12.1 I described the difficulties encountered with HbA1c measurement in the MDRS. FPG was measured in the MDRS. Substitution of FPG for HbA1c in both prevalence and progression analyses had little effect on the results (data not shown). Data from the MDRS demonstrate that measurement of HbA1c is feasible in the Malawian population and may be useful in assessment of glycaemic control in this region.

As described in Chapter 3, Section 3.5.4 the role of lipids in DR is incompletely understood. In the MDRS higher LDL cholesterol was associated with presence of STDR at baseline. However, no association was demonstrated between LDL cholesterol and DR progression. While lower levels of LDL cholesterol are linked to atherosclerosis [391] several studies have shown an association between raised LDL and DR severity [392,393]. Interestingly, some studies have found an effect of LDL level on maculopathy but not retinopathy [392]. This calls into question our analysis based on STDR: a term encompassing a composite threshold of retinopathy and maculopathy. A large number of lipoprotein subtypes are described. Both

apolipoprotein B (apoB) and non-high-density lipoprotein cholesterol (HDL-C) have been shown to be more accurate markers of cardiovascular risk than LDL cholesterol [394]. Modified forms of LDL such as malondialdehyde (MDA)-modified, and advanced glycation end (AGE)-product-modified LDL are thought to be important in chronic low grade vascular inflammation [395]. The role of malnutrition in complications of diabetes has not been widely studied, principally because of the low prevalence of under-nutrition in European and North American populations with diabetes. Particular lipid subgroups may be markers of good nutrition. If nutrient deficiencies contribute to microvascular disease then both low and high levels of particular lipids may be harmful adding further complexity to this area of interest.

In the MDRS main cohort sBP was associated with STDR at baseline but not progression of retinopathy. This is surprising due to the strong weight of evidence for the role of BP in DR progression from Western studies (reviewed in Chapter 3, Section 3.5.3). This area deserves further study in African populations. As expected baseline grade of DR was a strong determinant of DR progression at 24 months. In order to assess the effect of other variables without this strong determinant it would have been necessary to group subjects according to baseline grade of DR and produce a separate model for each group. In the case of the MDRS the numbers in each group would have been too small to adequately power each model.

11.8 Determinants of severity and progression: haemoglobin

In Chapter 6 I reported an association between prevalence of STDR in the main MDRS cohort and lower haemoglobin: a novel finding. In Chapter 8 (progression at 24 months) the proportion of subjects who were anaemic at baseline was greater in the subjects not assessed (died or lost to follow-up) than those who were seen. Haemoglobin was not significantly associated with 2 step progression at 24 months in either univariate or multivariate analysis. In the 2007 cohort (Chapter 9) 2 step progression at 5 years was associated with lower haemoglobin level. Data from the MDRS therefore supports an association of haemoglobin level with both severity and progression of DR.

Cross sectional (but not cohort) studies have demonstrated an association between presence of DR and anaemia in India [323-325] and China [187]. To my knowledge this relationship has not been demonstrated in an African population. The relationship between haemoglobin and diabetic nephropathy is complex because renal failure, from any cause, can lead to anaemia via decreased erythropoietin production. Renal anaemia is a marker for advanced nephropathy. Urine ACR, a sensitive marker for nephropathy, was included in my multivariate analyses. There is little data on associations between neuropathy in diabetes and anaemia although some evidence supports a role for autonomic neuropathy in abnormalities of erythropoietin regulation in type 2 diabetes [396]. It is tempting to hypothesise that the mechanism underlying an association between haemoglobin level and DR is impaired oxygen delivery and therefore increased oxygen stress at a microvascular level.

A potential confounder of the association between haemoglobin and retinopathy is socioeconomic status. Socioeconomic data were not collected in the MDRS. Anaemia may be a marker of nutritional status, renal disease (clinical or subclinical), comorbidities (anaemia of chronic disease) or poor general health. The aetiology of anaemia in SSA is multifactorial and includes deficiencies of micronutrients (e.g. iron, B12, folate); haemoglobinopathies; infections and chronic diseases (e.g., malaria, HIV, tuberculosis) [183]. To my knowledge the causes of anaemia in Malawian adults has not been studied. In a high-quality case control study of Malawian children severe anaemia was associated with bacteraemia, malaria, hookworm, HIV infection, glucose-6-phosphate dehydrogenase deficiency, vitamin A deficiency, and vitamin B12 deficiency [397]. Glucose-6-phosphate dehydrogenase deficiency is reported to increase risk of PDR in persons with type 1 diabetes [398]. The prevalence haemoglobinopathies in Malawian adults is not known but is thought to be low (S McKew, personal communication). Micronutrient deficiencies are potential therapeutic targets. Whether treatment of anaemia reduces diabetic microvascular complications is not known. Iron supplementation

has significant potential drawbacks in diabetes: both high iron level and iron supplementation have been associated with gestational diabetes [399,400].

11.9 Determinants of severity and progression: HIV

At baseline, 13.4% of subjects in the main MDRS cohort were HIV positive. HIV infection was negatively associated with presence of STDR in univariate but not multivariate analysis. At 24 months HIV infection was negatively associated with 2 step progression in multivariate analysis. Mortality was higher in subjects with HIV than in the cohort as a whole. In the 2007 cohort (Chapter 9) no association between HIV infection and 2 step progression at 5 years was demonstrated. 5 year mortality in the 2007 cohort could not be reliably determined. However, the proportion of subjects with HIV was not significantly different between subjects seen in 2012 and those who were not seen. Data from the MDRS supports a negative association between HIV infection and severity and progression of DR: a novel finding.

To my knowledge no previous study has investigated the relationship of HIV with DR. HIV can directly affect the kidney leading to HIV associated nephropathy (HIVAN) [173]. Proteinuria is the presenting feature of this condition. HIV and ART are associated with peripheral neuropathy. Therefore, any association between HIV and diabetic microvascular complications except DR will be difficult to investigate. Both HIV infection and anti-retroviral therapies (ART) are associated with a vasculopathy which manifests as increased cardiovascular and cerebrovascular risk [326,327]. It is possible that HIV and ART affect DR pathophysiology via multiple effects on cardiometabolic traits. In African populations HIV (when adjusted for ART exposure) is associated with lower mean BMI, lower systolic and diastolic BP, higher mean triglyceride levels, lower mean HDL and lower mean LDL [401]. ART is associated with raised LDL and HDL but lower triglycerides [401]. HIV is weakly associated with raised HbA1c while ART is associated with lower HbA1c [401]. Chronic low grade inflammation is important in the pathophysiology of DR. HIV is generally seen as a pro-inflammatory state. However, it is conceivable that the

effect of HIV on cytokines and other inflammatory mediators as well as leucocytes and associated inflammatory cells may affect progression of DR.

An important potential confounder of the association between HIV and DR is early diagnosis of diabetes in HIV positive subjects. Patients attending medical facilities for ART treatment may be more likely to be tested for diabetes than the general population. While the logistic regression analyses presented above controlled for known duration of diabetes, the duration of diabetes before diagnosis was not known. A potential source of bias is data censoring due to higher mortality in HIV infected subjects.

11.10 Determinants of severity and progression: implications

The observational data presented in this thesis will facilitate design of appropriate intervention studies in the region. The magnitude of effect size for variables such as glycaemic control is important information required for accurate cost-effectiveness studies and cannot be assumed from studies in Europe and North America. Ethical concerns about such trials could be allayed by intelligent study design; one example is the stepped wedge study design. Case detection of retinopathy in persons with diabetes along the UK model will not be cost effective in SSA. It is likely that targeted screening based on determinants of DR severity and progression will be employed. A risk model based on data from the MDRS and similar studies could aid effective allocation of resources. Epidemiological data from the MDRS has implications for our understanding of DR pathophysiological. Mechanisms of disease are explored in the next section in the context of results from the case-control study of endothelial function.

11.11 Implications of endothelial function studies

Data from the MDRS case-control study of endothelial function are compared to previous studies of endothelial function in diabetes in Chapter 10, Section 10.6.2, to studies of endothelial function and microvascular disease in diabetes in Section 10.6.3, to studies of endothelial function and mortality in diabetes in Section 10.6.4,

and to studies of microvascular reactivity in diabetes in Section 10.6.5. Conclusions which can be drawn from the MDRS are restricted by the limitations outlined in Section 10.6.6. Results from my studies add to the literature on endothelial dysfunction as an important pathological mechanism in DR including in Malawian subjects. The majority of work on endothelial dysfunction in diabetes has been performed in the developed world. I have provided evidence of endothelial dysfunction in diabetes in a different population.

Future work on endothelial function in subjects with diabetes could include investigation of other endothelial biomarkers including angiopoietins, endothelial microparticles and asymmetric dimethyl L-arginine (ADMA). Analysis of the activation status of circulating leucocyte sub-classes by fluorescence-activated cell sorting (FACS) would complement studies of the vascular endothelium. Direct endothelial cell histology and/or culture following sub-cutaneous fat biopsy is more invasive but would allow laboratory based study of peripheral endothelium. There is clear evidence for an association between acute episodes of infective disease and ischaemic vascular events. For example, between mycoplasma pneumonia and stroke [402] and between influenza and stroke [403]. In malaria, episodes of acute infection may lead to chronic endothelial activation and alterations in the coagulation pathway [370]. Investigation of a role for acute infection (e.g. malaria) and endothelial dysfunction in the context of DR would be extremely interesting and relevant to subjects with diabetes in Malawi.

11.12 Limitations of the work presented in this thesis

Specific limitations of the various component studies of the MDRS are discussed in the following chapters and summarised here: Chapter 6, Section 6.5.6; Chapter 8, Section 8.5.3; Chapter 9, Section 9.5.2; and Chapter 10, Section 10.6.6.

Missing data The proportion of data in the MDRS which was missing was very small. Data on a number of potentially interesting variables were not recorded. I explored the possibility of quantifying the burden of malaria in the MDRS cohort. Measuring parasitaemia during acute infection is readily available and cheap. However, this

measure tells one little about the burden of malaria over months or years. Measurement of anti-malarial antibodies is possible but was prohibitively expensive. Socioeconomic data was not collected in the MDRS but is being collected for participants in the 'MDRS 2' (described in Section 11.14 below).

Limitations of specific tests No test is 100% sensitive and specific. Grading of retinal photographs at an accredited reading centre (dual grading with arbitration) is the current reference standard for DR classification in the context of research and systematic screening. A small number of subjects were ungradeable on photography but gradeable on slit-lamp biomicroscopy; reliance on clinical examination was unavoidable in these circumstances. In each round of assessment less than 1% of subjects were ungradeable on both photography and slit-lamp biomicroscopy. Biochemical tests performed at the Wellcome Trust Clinical Research Programme Laboratories are subject to internal and external quality control. Specific issues encountered with measurement of HbA1c are discussed in Chapter 5 Section 5.12.1.

Cohort study approach Cohort studies are expensive and of long duration. A major advantage of such studies is that exposure (determinants of severity and progression) is measured before disease onset thereby reducing bias in terms of disease development. The cohort approach permits study of multiple outcomes such as DR progression and mortality. The results of cohort studies must be interpreted in the context of other research, usually including ecological and case-control studies. The reference standard for validity is an intervention study to reduce/eliminate exposure to a particular variable. For some variables such as age or HIV infection randomised interventions are not possible.

A potential criticism of our methodology is the definition used for any DR: any haemorrhage or microaneurysm in either eye, and our assumption in analysis of a linear progression of DR. Haemorrhages and microaneurysms are not specific for DR and may be observed in the absence of diabetes. Wong et al [404] examined 7992 adults aged 49-73 without diabetes. 4.8% of subjects had retinal changes

indistinguishable from DR. After 3 years follow-up the number of subjects progressing to diabetes did not differ between the groups with or without retinal changes. Cugati et al [110] analysed data from the Blue Mountains Eye Study. In 1725 subjects without diabetes or retinopathy (microaneurysms, haemorrhages, hard or soft exudates) at baseline the 5-year cumulative incidence of retinopathy was 9.7% (95% CI 8.3-11.1). Of subjects with retinal signs but no diabetes at baseline, 3.5% went on to develop diabetes. 13.3% of retinal changes present at baseline progressed but 72.3% regressed or disappeared over the course of the study. Therefore there is an increasing body of evidence that the relationship between blood glucose, pre-diabetes, clinical diabetes, hypertension, age and detectable retinal changes is more complex than previously thought.

Case-control approach Endothelial function studies were conducted using a case-control design. A longitudinal analysis was also performed. Case-control studies are prone to selection bias of both cases and controls. While we excluded diabetes in our control subjects according to ADA criteria [123] and produced robust phenotyping for cases and controls, it is possible that unmeasured confounders such as exposure to environmental factors including domestic smoke pollution and foodstuffs could have influenced our results. Selecting a list of candidate markers of endothelial dysfunction on the basis of previous research is a logical approach. However, in the absence of a widely accepted definition of endothelial dysfunction and an incomplete understanding of the relationship between endothelial pathology and diabetic microvascular complications, it is difficult to draw conclusions from our results when few strong associations are recorded.

11.13 Ongoing research arising directly from this work

Further analysis of the MDRS dataset The MDRS has produced a large dataset on which further analysis is possible. The UKPDS outcomes model [405] is a computer simulation model based on patient data from the UKPDS. It is designed to predict estimated life expectancy and quality adjusted life expectancy for each member of a

given population. MDRS data could be used to assess the performance of the UKPDS outcomes model in a population with diabetes in SSA.

A number of methods have been used to estimate the delay between onset and diagnosis of type 2 diabetes from prevalence studies of DR. Using a simple linear model Ellis et al [193] plotted prevalence of any retinopathy with duration of known disease using data from the Prevalence of Diabetic Eye Disease in Scotland study. In this model the 'x' axis intercept gives the estimate of mean delay in clinical diagnosis of diabetes. This approach has been criticised for using a simplistic linear model and for including stages of retinopathy not specific to diabetes. Porta et al [406] examined data from 2 groups of subjects corresponding to type 1 and type 2 diabetes. Time for retinopathy to develop after diagnosis of diabetes was defined as the mean time for development of 'moderate DR' (study specific definition) in subjects with type 1 diabetes. Using a quadratic model the authors estimated this figure to be 3.29 years in their study population. A linear model was used to correlate known duration of diabetes with prevalence of 'moderate DR' in subjects with type 2 diabetes. The authors extrapolated this model to estimate time from appearance of retinopathy to diagnosis of type 2 diabetes. A figure of 2.66 years was reported. Addition of these figures gives an estimate of 6.05 years between onset and diagnosis of type 2 diabetes [406]. A similar analysis would be possible with MDRS data.

'MDRS 2' In 2013 a grant from the British Council for the Prevention of Blindness was secured by my colleagues and I to investigate the effectiveness and cost-effectiveness of laser treatment for DR in Malawi. The effectiveness of laser treatment in Africa is assumed from landmark studies in resource-rich countries without taking account of confounders such as late presentation, HIV, malaria, malnutrition episodes and treatment non-availability. The 'MDRS 2' is a prospective cohort study which is continuing follow-up of patients that I treated with laser as part of the MDRS study and also recruiting new subjects with DR requiring laser treatment. Subjects will be assessed at 24 and 72 months with regard to visual acuity and development or regression of proliferative DR. Results will be compared

for non-inferiority with data from the recent Diabetic Retinopathy Clinical Research Network (DRCR.net) [407] and other published data.

11.14 Future studies

Effectiveness and cost-effectiveness of systematic diabetic retinopathy screening in Europe is well established. Systematic screening is cost-effective for sight years preserved compared with no screening. Variation in age of onset of diabetes, glycaemic control, sensitivity of the screening test and compliance rates influence the cost-effectiveness of screening programs [408]. Digital photography, as used by the English National Screening Programme [409], with the addition of telemedicine links has the potential to deliver cost-effective, accessible screening to rural and remote populations. In Europe a strong evidence base has driven the political agenda for service development for diabetes and its complications. Further high-quality research on the effectiveness and cost-effectiveness of DR case detection and management models tailored to local needs is necessary. This evidence should effect change in national policies and transform services and outcomes.

My colleagues in the Department of Eye and Vision Science at the University of Liverpool and I will shortly submit a grant proposal to investigate the prevention of avoidable visual loss from DR in Malawi by developing a targeted screening programme. Specifically we intend to investigate perceived barriers to DR care in Malawi, to evaluate implementation of a model of DR screening appropriate to local facilities and resource constraints, and to introduce DR screening into representative central and district hospital diabetes clinics. We propose to add a call/recall system to the existing database of patients with diabetes at QECH, Blantyre. We intend to use a newly developed low-cost portable fundus camera (VisionQuest) to capture retinal images. Ophthalmic Clinical Officers (OCOs) and opticians will be trained in fundus photography and OCOs will receive training in image grading and referral guidelines.

Having produced an effective and achievable screening model in QECH it will be implemented in representative central and district hospitals in a step-wise fashion. Data will be collected on the numbers of patients screened, number of screen positives (referable retinopathy), sensitivity and specificity of the screening test (true and false positives and negatives) against a reference standard of clinical examination by an ophthalmologist, proportion of patients unable to be screened by photography, number of patients treated with laser and costings. A cost benefit analysis will be performed. Building on our work on the cost-effectiveness of laser treatment in Malawi, data collected during the study will be used to develop a Markov model of DR screening in SSA. Model computation will explore the cost-effectiveness of the programme and the limits on sustainability of screening and care in a low-resource setting. We aim to establish an achievable and cost effective DR screening model which is generalisable to regional ophthalmology units and district hospitals with diabetes, NCD or vascular risk clinics across sub-Saharan Africa (SSA).

11.15 Summary

The MDRS is the first prospective cohort study of DR in Sub-Saharan Africa. This programme of research has reported prevalence, incidence and progression of DR in patients attending diabetes clinics in Southern Malawi: vital data for health policy makers in the region. Observational data regarding determinants of severity and progression of DR presented in this thesis (including novel associations) will facilitate design of appropriate intervention studies in the region and provide insights into the pathophysiology of DR. My hope is that, building on the MDRS, current and future studies will provide an evidence base for cost-effective DR case detection and management which is achievable in a low resource setting and generalisable across sub-Saharan Africa.

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APPENDICES

Appendix 1. Grading definitions used in the ETDRS

Definitions are taken from the ETDRS report 10 [86].

Definitions of present, absent, questionable and cannot grade

Present: Grader is greater than 90% certain that feature is present

Absent: Grader is less than 50% certain that the feature is present

Questionable: Recorded for a characteristic when a grader is 50 to 90% certain that it is present. When an abnormality is definitely present but its nature is uncertain the grader assigns the grade 'questionable' for the characteristic considered to be most likely and 'absent' for the one(s) considered less likely. For example, when an abnormality is believed to be either new vessels or IRMA, but is more likely the former, the grader assigns the grade of questionable for new vessels and absent for IRMA. Only if the grader favours neither characteristic over the other is a grade of questionable assigned to both.

Cannot grade: Grader is unable to evaluate or distinguish with more than 50% confidence the presence or absence of a feature and more than 75% of the image is obscured i.e. poor image quality.

Definitions of clinical features of retinopathy

Microaneurysm: Defined as a red spot less than 125µm in its longest dimension (approximately the width of a vein at the disc margin) with sharp margins.

Haemorrhage: Defined as either a red spot less than 125µm with irregular margins or a red lesion greater than 125µm (unless it is clear that it is a microaneurysm).

Cotton wool spot (CWS): Superficial white, pale yellow-white or greyish white lesion with ill-defined (feathery) edges.

Venous abnormalities: Three abnormalities are assessed separately.

- (i) Venous beading (VB): localised increases in venous calibre which sometimes resemble a string of beads

- (ii) Venous narrowing: localised narrowing of venous calibre
- (iii) Venous loops and / or reduplication. A venous loop is an abrupt, curving deviation of a vein from its normal path. Reduplication of a vein is the dilatation of a pre-existing channel or the proliferation of a new channel adjacent to original vein.

Intra-retinal microvascular abnormalities (IRMA): Tortuous intraretinal vascular segments, varying in calibre from barely visible to 31 μm (approximately one fourth the width of a major vein at the disc margin).

New vessels at the disc (NVD): New vessels that are clearly located on the surface of the retina (not within the retina) or further forward in the vitreous cavity on the disc or within 1DD of its margin.

New vessels elsewhere (NVE): New vessels that are clearly located on the surface of the retina (not within the retina) or further forward in the vitreous cavity except for those on the disc or within 1DD of its margin.

Fibrous proliferations at the disc: Fibrous tissue opaque enough to be definitely seen, with or without accompanying new vessels, on the disc or within 1DD of its margin.

Fibrous proliferations at the disc: Fibrous tissue opaque enough to be definitely seen, with or without accompanying new vessels, except for those on the disc or within 1DD of its margin.

Pre-retinal haemorrhage (PRH): Haemorrhage just anterior to the retina or under its internal limiting membrane. Both boat shaped haemorrhages with a fluid level and round, oval or linear patches are included.

Vitreous haemorrhage: Haemorrhage further forward in the vitreous cavity than PRH.

Hard exudates: Small white or yellowish-white deposits with sharp margins. Often have a slightly waxy or glistening appearance. Usually located in the outer layers of the retina.

Retinal thickening (oedema): Thickening of the retina (with or without partial loss of transparency).

Drusen: Deep yellowish white dots, sometimes circumscribed by a thin line of pigment.

Appendix 2. Search histories for systematic review

Search histories for the electronic databases Medline (PubMed), EMBASE, Web of Science, African index Medicus and OpenSigle.

Search history Pubmed:

- #1 Search "Diabetic Retinopathy"[Mesh]
- #2 Search Diabetic maculopathy ti, ab
- #3 Search diabet* AND ((macular edema) OR (macular oedema))
- #4 Search #1 OR #2 OR #3
- #5 Search ("Africa"[MeSH] OR Africa*[tw] OR Algeria[tw] OR Angola[tw] OR Benin[tw] OR Botswana[tw] OR "Burkina Faso"[tw] OR Burundi[tw] OR Cameroon[tw] OR "Canary Islands"[tw] OR "Cape Verde"[tw] OR "Central African Republic"[tw] OR Chad[tw] OR Comoros[tw] OR Congo[tw] OR "Democratic Republic of Congo"[tw] OR Djibouti[tw] OR Egypt[tw] OR "Equatorial Guinea"[tw] OR Eritrea[tw] OR Ethiopia[tw] OR Gabon[tw] OR Gambia[tw] OR Ghana[tw] OR Guinea[tw] OR "Guinea Bissau"[tw] OR "Ivory Coast"[tw] OR "Cote d'Ivoire"[tw] OR Jamahiriya[tw] OR Jamahirya[tw] OR Kenya[tw] OR Lesotho[tw] OR Liberia[tw] OR Libya[tw] OR Libia[tw] OR Madagascar[tw] OR Malawi[tw] OR Mali[tw] OR Mauritania[tw] OR Mauritius[tw] OR Mayote[tw] OR Morocco[tw] OR Mozambique[tw] OR Mocambique[tw] OR Namibia[tw] OR Niger[tw] OR Nigeria[tw] OR Principe[tw] OR Reunion[tw] OR Rwanda[tw] OR "Sao Tome"[tw] OR Senegal[tw] OR Seychelles[tw] OR "Sierra Leone"[tw] OR Somalia[tw] OR "South Africa"[tw] OR "St Helena"[tw] OR Sudan[tw] OR Swaziland[tw] OR Tanzania[tw] OR Togo[tw] OR Tunisia[tw] OR Uganda[tw] OR "Western Sahara"[tw] OR Zaire[tw] OR Zambia[tw] OR Zimbabwe[tw] OR "Central Africa"[tw] OR "Central African"[tw] OR "West Africa"[tw] OR "West African"[tw] OR "Western Africa"[tw] OR "Western African"[tw] OR "East Africa"[tw] OR "East African"[tw] OR "Eastern Africa"[tw] OR "Eastern African"[tw] OR "North Africa"[tw] OR "North African"[tw] OR "Northern Africa"[tw] OR "Northern African"[tw] OR "South African"[tw] OR "Southern Africa"[tw] OR "Southern African"[tw] OR "sub Saharan Africa"[tw] OR "sub Saharan African"[tw] OR "subSaharan Africa"[tw] OR "subSaharan African"[tw]) NOT ("guinea pig"[tw] OR "guinea pigs"[tw] OR "aspergillus niger"[tw])
- #6 Search #4 And #5
- #7 Limit #6 to Human

Search history EMBASE:

- 1 diabetic retinopathy.mp. or diabetic retinopathy/
- 2 diabetic macular edema/ or retina maculopathy/ or retina macula edema/ or diabetic maculopathy.mp.
- 3 (diabet* and (macul* edema or macul* oedema)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer]
- 4 (diabet* and macul*).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer]
- 5 1 or 2 or 3 or 4
- 6 (Africa or Africa* or Algeria or Angola or Benin or Botswana or Burkina Faso or Burundi or Cameroon or Canary Islands or Cape Verde or Central African Republic or Chad or Comoros or Congo or Democratic Republic of Congo or Djibouti or Egypt or Equatorial Guinea or Eritrea or Ethiopia or Gabon or Gambia or Ghana or Guinea or Guinea Bissau or Ivory Coast or (Cote and Ivoire) or Jamahiriya or Jamahiryia or Kenya or Lesotho or Liberia or Libya or Libia or Madagascar or Malawi or Mali or Mauritania or Mauritius or Mayote or Morocco or Mozambique or Mocambique or Namibia or Niger or Nigeria or Principe or Reunion or Rwanda or Sao Tome or Senegal or Seychelles or Sierra Leone or Somalia or South Africa or St Helena or Sudan or Swaziland or Tanzania or Togo or Tunisia or Uganda or Western Sahara or Zaire or Zambia or Zimbabwe or Central Africa or Central African or West Africa or West African or Western Africa or Western African or East Africa or East African or Eastern Africa or Eastern African or North Africa or North African or Northern Africa or Northern African or South African or Southern Africa or Southern African or South Africa).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer]
- 7 5 and 6
- 8 Limit 7 to Humans

Search history Web of Science:

(Diabetic retinopathy) or (Diabetic maculopathy) or ((diabet* AND ((macular edema) OR (macular oedema)) [Topic]

AND

(Africa or Africa* or Algeria or Angola or Benin or Botswana or Burkina Faso or Burundi or Cameroon or Canary Islands or Cape Verde or Central African Republic or Chad or Comoros or Congo or Democratic Republic of Congo or Djibouti or Egypt or Equatorial Guinea or Eritrea or Ethiopia or Gabon or Gambia or Ghana or Guinea or Guinea Bissau or Ivory Coast or (Cote and Ivoire) or Jamahiriya or Jamahiryia or Kenya or Lesotho or Liberia or Libya or Libia or Madagascar or Malawi or Mali or Mauritania or Mauritius or Mayote or Morocco or Mozambique or Mocambique or Namibia or Niger or Nigeria or Principe or Reunion or Rwanda or Sao Tome or Senegal or Seychelles or Sierra Leone or Somalia or South Africa or St Helena or Sudan or Swaziland or Tanzania or Togo or Tunisia or Uganda or Western Sahara or Zaire or Zambia or Zimbabwe or Central Africa or Central African or West Africa or West African or Western Africa or Western African or East Africa or East African or Eastern Africa or Eastern African or North Africa or North African or Northern Africa or Northern African or South African or Southern Africa or Southern African or South Africa) [topic]

Search history African index Medicus, OpenSigle:

(Diabetic retinopathy) or (Diabetic maculopathy) or ((diabet* AND ((macular edema) OR (macular oedema))

Appendix 3A. Patient information document 1



THE COLLEGE OF MEDICINE
Malawi-Liverpool-Wellcome Trust
Clinical Research Programme

www.mlw.medcol.mw

PATIENT INFORMATION DOCUMENT 1

Date 4th October 2011
Version 02 (English)

STUDY TITLE: DETERMINANTS OF DIABETIC RETINOPATHY SEVERITY AND PROGRESSION IN MALAWI

INTRODUCTION

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your family doctor if you wish. Ask us if there is anything that is not clear or if you would like more information.

WHAT IS THE PURPOSE OF THE STUDY?

Diabetes can cause damage to blood vessels at the back of the eye (diabetic eye disease) which can lead to loss of vision. This study has three key aims:

1. To investigate how many patients are affected by diabetic eye disease and how quickly the condition progresses in Southern Malawi
2. To investigate the affect of factors such as blood sugar level, blood pressure and HIV infection on severity and progression of diabetic eye disease
3. To investigate the function of the thin layer of cells lining the inner surface of blood vessels (the endothelium) in patients with diabetes and relationships with diabetic eye disease

The information we get from this study will help us to improve management of future patients with diabetic eye disease. The study has been planned by Dr Philip Burgess (Eye Doctor, Queen Elizabeth Central Hospital and Liverpool, UK), Professor Theresa Allain (Medical Doctor, Queen Elizabeth Central Hospital), and Professor Simon Harding (Eye Doctor, Liverpool, UK). The research is funded by the 'Wellcome Trust', a global charitable foundation.

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WHY AM I BEING ASKED TO TAKE PART?

You are being asked to take part because you have diabetes or because you have been selected as a 'control' subject (someone without diabetes).

WHAT WILL HAPPEN TO ME?

The study will include 340 people with diabetes. All patients will be asked to participate in **Part A** of the study. On the day of your diabetes clinic appointment a nurse will ask you questions about your health and you will have a brief physical examination (including blood pressure, height and weight, and vision). Drops to dilate your pupils will be put into your eyes and photographs will be taken of the back of your eyes. Blood tests will be taken including a measure of your blood sugar, red blood cells and an HIV test (volume of blood to be taken 20mls - approximately 4 teaspoons). You will be asked for a urine sample.

These tests will take about an hour. You will be invited to return to repeat the tests in 12 months and then 24 months at the time of a diabetes clinic appointment. Food will be provided at each visit and your transport costs will be reimbursed. If the tests show that you have treatable eye disease you will be referred to the eye clinic for treatment (e.g. LASER therapy for diabetic eye disease, surgery for cataract). If you test positive for HIV you will be referred to the HIV clinic for treatment.

Of the 340 patients taking part in the study, 160 will also be invited to participate in **Part B**. If you take part some additional tests will be performed on your blood samples which measure the health of blood vessels. A test of blood vessel function will also be performed - this involves taking measurements with a probe on your finger whilst wearing a blood pressure cuff on your arm for 5 minutes. These tests will be performed only once.



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WHAT WILL HAPPEN TO MY INFORMATION AND SAMPLES?

All information which is collected about you during the course of the research will be kept strictly confidential. If you consent to take part in the research your medical records will be inspected by members of the research team for purposes of analysing the results. Your records may also be viewed by sponsors and monitors of the research to ensure that the study is being carried out in an acceptable manner. Blood samples will be analysed at the time and will also be stored at the Malawi-Liverpool-Wellcome Trust laboratories at Queen Elizabeth Central Hospital for future analysis. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it.

WHAT IMPACT WILL THE STUDY HAVE ON ME?

Taking part in the study has potential benefits and risks for you.

BENEFITS If you participate in the study you will have blood tests including a test of your diabetes control and the fats in your blood not otherwise available in the diabetes clinic. Test results will be fed back to doctors in the clinic allowing them to more effectively monitor your diabetes. You will also have a thorough examination of the back of the eye which is not always available at the diabetes clinic. This examination has the potential to pick up diabetic eye disease at an early stage which may benefit from LASER treatment. If you are a control subject (do not have diabetes) different benefits apply to you: you will be tested for diabetes, high blood pressure and HIV. If you have any of these diseases you will be referred for treatment.

RISKS The study will require your time (3 visits of 1 hour on top of usual clinic visit). The study tests may reveal a disease of which you were unaware (e.g. HIV infection). This could cause distress but would allow you to access treatment at an earlier stage.

It is very unlikely that you are harmed by taking part in this research project. If you are harmed, indemnity cover for the study is provided by the University of Liverpool who will be liable for adverse events caused by the study in research participants. If you wish to complain about any aspect of the way you have been approached or treated during the course of this study, you can do so through

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MLW Clinical research programme or the Department of Medicine, Queen Elizabeth Central Hospital (addresses below). Sometimes during the course of a research project, new information becomes available about the disease or tests that are being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study.

WILL I FIND OUT ABOUT THE STUDY RESULTS?

The results of the research will be published approximately 3 years from the start of the study. You may ask for a copy of the study report or for a verbal explanation from a doctor/nurse at the diabetes clinic. You will not be identified by name or photograph in any report/publication.

WHAT HAPPENS IF I DON'T WANT TO TAKE PART?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive. Similarly, if you decide not to participate this will not affect the standard of care you receive.

WHO CAN I GO TO FOR MORE INFORMATION ABOUT THIS?

For questions about the research, participant's rights, research-related injury or if you wish to complain about any aspect of the study you can contact:

1. Dr. Philip Burgess, MLW Research Programme, Queen Elizabeth Central Hospital, College of Medicine, PO Box 30096, Chichiri, Blantyre. Telephone: 01874628

2. Professor Theresa Allain, Department of Medicine, QECH, Chichiri, Blantyre. Telephone: 01871911

You may also contact the Ethics committee who have approved the study directly:

College of Medicine Research and Ethics Committee (COMREC), College of Medicine, P/Bag 360, Chichiri, Blantyre 3. Telephone: 01 877 245

Appendix 3B. Patient information document 2



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PATIENT INFORMATION DOCUMENT 2

Date 4th Oct 2011
Version 02 (English)

STUDY TITLE: DETERMINANTS OF DIABETIC RETINOPATHY SEVERITY AND PROGRESSION IN MALAWI

INTRODUCTION

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your family doctor if you wish. Ask us if there is anything that is not clear or if you would like more information.

WHAT IS THE PURPOSE OF THE STUDY?

Diabetes can cause damage to blood vessels at the back of the eye (diabetic eye disease) which can lead to loss of vision. You may remember that your eyes were examined around 5 years ago as part of a research study. Our team wish to examine your eyes again in order to determine to what extent (if at all) the condition has got worse over this time. The information we get from this study will help us to improve management of future patients with diabetic eye disease. The study has been planned by Dr Philip Burgess (Eye Doctor, Queen Elizabeth Central Hospital and Liverpool, UK), Professor Theresa Allain (Medical Doctor, Queen Elizabeth Central Hospital), and Professor Simon Harding (Eye Doctor, Liverpool, UK). The research is funded by the 'Wellcome Trust', a global charitable foundation.

WHAT WILL HAPPEN TO ME?

All of the 281 patients who were examined 5 years ago will be invited to participate in the study. On the day of your diabetes clinic appointment a nurse will ask you questions about your health and you will have a brief physical examination (including blood pressure, height and weight, and vision). Drops to dilate your pupils will be put into your eyes and photographs will be taken of the back of

Determinants of DR severity and progression, Patient information document 2, Version 02 (English), 04/10/11



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your eyes. Blood tests will be taken including a measure of your blood sugar, red blood cells and an HIV test (volume of blood to be taken 20mls - approximately 4 teaspoons of blood). You will be asked for a urine sample. These tests will take about an hour. Food will be provided and your transport costs will be reimbursed. If the tests show that you have treatable eye disease you will be referred to the eye clinic for treatment (e.g. LASER therapy for diabetic eye disease, surgery for cataract). If you test positive for HIV you will be referred to the HIV clinic for treatment.

WHAT WILL HAPPEN TO MY INFORMATION AND SAMPLES?

All information which is collected about you during the course of the research will be kept strictly confidential. If you consent to take part in the research your medical records will be inspected by members of the research team for purposes of analysing the results. Your records may also be viewed by sponsors and monitors of the research to ensure that the study is being carried out in an acceptable manner. Blood samples will be analysed at the time and will also be stored at the Malawi-Liverpool-Wellcome Trust laboratories at Queen Elizabeth Central Hospital for future analysis. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it.

WHAT IMPACT WILL THE STUDY HAVE ON ME?

Taking part in the study has potential benefits and risks for you.

BENEFITS If you participate in the study you will have blood tests including a test of your diabetes control and the fats in your blood not otherwise available in the diabetes clinic. Test results will be fed back to doctors in the clinic allowing them to more effectively monitor your diabetes. You will also have a thorough examination of the back of the eye which is not always available at the diabetes clinic. This examination has the potential to pick up diabetic eye disease at an early stage which may benefit from LASER treatment.

RISKS The study will require your time (1 hour on top of your usual clinic visit). The study tests may reveal a disease of which you were unaware (e.g. HIV infection). This could cause distress but would allow you to access treatment at an earlier stage. It is very unlikely that you are harmed by taking

Determinants of DR severity and progression, Patient information document 2, Version 02 (English), 04/10/11



part in this research project. If you are harmed, indemnity cover for the study is provided by the University of Liverpool who will be liable for adverse events caused by the study in research participants. If you wish to complain about any aspect of the way you have been approached or treated during the course of this study, you can do so through MLW Clinical Research Programme or the Department of Medicine (addresses below).

WILL I FIND OUT ABOUT THE STUDY RESULTS?

We plan to publish the results of the research within 1 year from the start of the study. You may ask for a copy of the study report or for a verbal explanation from a doctor/nurse at the diabetes clinic. You will not be identified by name or photograph in any report/publication.

WHAT HAPPENS IF I DON'T WANT TO TAKE PART?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive. Similarly, if you decide not to participate this will not affect the standard of care you receive.

WHO CAN I GO TO FOR MORE INFORMATION ABOUT THIS?

For questions about the research, participant's rights, research-related injury or if you wish to complain about any aspect of the study you can contact:

1. Dr. Philip Burgess, MLW Research Programme, Queen Elizabeth Central Hospital, College of Medicine, PO Box 30096, Chichiri, Blantyre. Telephone: 01874628

2. Professor Theresa Allain, Department of Medicine, QECH, Chichiri, Blantyre. Telephone: 01871911

You may also contact the Ethics committee who have approved the study directly:

College of Medicine Research and Ethics Committee (COMREC), College of Medicine, P/Bag 360, Chichiri, Blantyre 3. Telephone: 01 877 245

Appendix 3C. Patient information document 3



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PATIENT INFORMATION DOCUMENT 3

Date 20th April 2012
Version 01 (English)

STUDY TITLE: DETERMINANTS OF DIABETIC RETINOPATHY SEVERITY AND PROGRESSION IN MALAWI

INFORMATION ON HIV TESTING IN THIS STUDY

Thank you for taking part in this research study. One of the aims of this research is to investigate the affect of factors such as blood sugar level, blood pressure and HIV infection on severity and progression of diabetic eye disease. For this reason you will be offered Voluntary Testing and Counselling for HIV (VCT). As well as being beneficial for our research, knowledge of your HIV status can be useful for you. It allows you to make informed decisions about you and your partner's health. In the event that your test is positive (reactive) you may find this information distressing but you may be able to access treatment at an earlier stage than had you not known.

WHAT IF I DO NOT WISH TO KNOW MY HIV STATUS?

If you do not wish to know your HIV status you will be offered the opportunity for your samples to be tested for HIV infection in the laboratory. The results of the test will not be disclosed to you. Therefore the research team can investigate the affects of HIV on diabetic eye disease but you will avoid knowing the result. If you wish to do this you will be asked to sign an additional consent form (Consent form 3).

WHAT HAPPENS IF I DON'T WANT TO BE TESTED FOR HIV AT ALL?

It is up to you to decide whether or not you wish your samples to be tested for HIV. If you do not wish your samples to be tested even without knowing the results you can simply indicate this to the study team and on the study consent forms. You do not have to give a reason for your decision and this will not affect the standard of care you receive.



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WHAT WILL HAPPEN TO MY INFORMATION AND SAMPLES?

All information which is collected about you during the course of the research will be kept strictly confidential. Blood samples will be analysed at the time and will also be stored at the Malawi-Liverpool-Wellcome Trust laboratories at Queen Elizabeth Central Hospital for future analysis. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it.

WHO CAN I GO TO FOR MORE INFORMATION ABOUT THIS?

For questions about the research, participant's rights, research-related injury or if you wish to complain about any aspect of the study you can contact:

1. Dr. Philip Burgess, MLW Research Programme, Queen Elizabeth Central Hospital, College of Medicine, PO Box 30096, Chichiri, Blantyre. Telephone: 01874628
2. Professor Theresa Allain, Department of Medicine, QECH, Chichiri, Blantyre. Telephone: 01871911

You may also contact the Ethics committee who have approved the study directly:

College of Medicine Research and Ethics Committee (COMREC), College of Medicine, P/Bag 360, Chichiri, Blantyre 3. Telephone: 01 877 245

Appendix 4A. Consent form 1A



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Centre	Study Number	Patient Identification Number

PATIENT OR GUARDIAN CONSENT FORM 1A

DETERMINANTS OF DIABETIC RETINOPATHY SEVERITY AND PROGRESSION IN MALAWI

Statement by patient. Please circle Yes or No to confirm whether you agree or do not agree with each of the points below:

- I have read the information sheet or it has been read to me Yes / No
- I have had the chance to ask questions and I am satisfied with the answers Yes / No
- I voluntarily agree to take part in Part A of the study but I know that I can change my mind at a later date Yes / No
- I voluntarily agree that my blood samples and personal information can be collected and stored for the purpose of this study
(volume of blood to be taken 20mls - approximately 4 teaspoons) Yes / No
- I voluntarily agree to testing for HIV infection Yes / No

Name of participant	Date	Signature
*Name of guardian	Date	Signature
**Name of witness	Date	Signature
Name of staff administering consent	Date	Signature

*If participant cannot give consent on their own e.g. mentally ill **If both participant and guardian cannot read or write.

Appendix 4B. Consent form 1B



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Centre	Study Number	Patient Identification Number

PATIENT OR GUARDIAN CONSENT FORM 1B

DETERMINANTS OF DIABETIC RETINOPATHY SEVERITY AND PROGRESSION IN MALAWI

This consent form refers to participation in the sub-study Part B. Please circle Yes or No to confirm whether you agree or do not agree with each of the points below:

- I have read the information sheet or it has been read to me Yes / No
- I have had the chance to ask questions and I am satisfied with the answers Yes / No
- I voluntarily agree to take part in Part B of the study (extra tests performed on my blood sample and a test of blood vessel function) but I know I can change my mind at a later date Yes / No
- I voluntarily agree that my blood samples and personal information can be collected and stored for the purpose of this study Yes / No

Name of participant	Date	Signature
*Name of guardian	Date	Signature
**Name of witness	Date	Signature
Name of staff administering consent	Date	Signature

*If participant cannot give consent on their own e.g. mentally ill **If both participant and guardian cannot read or write.

Appendix 4C. Consent form 2



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Centre	Study Number	Patient Identification Number

PATIENT OR GUARDIAN CONSENT FORM 2

DETERMINANTS OF DIABETIC RETINOPATHY SEVERITY AND PROGRESSION IN MALAWI

Statement by patient. Please circle Yes or No to confirm whether you agree or do not agree with each of the points below:

- I have read the information sheet or it has been read to me Yes / No
- I have had the chance to ask questions and I am satisfied with the answers Yes / No
- I voluntary agree to take part in study but I know that I can change my mind at a later date Yes / No
- I voluntary agree that my blood samples and personal information can be collected and stored for the purpose of this study (volume of blood to be taken 20mls - approximately 4 teaspoons) Yes / No
- I voluntary agree to testing for HIV infection Yes / No

Name of participant	Date	Signature
*Name of guardian	Date	Signature
**Name of witness	Date	Signature
Name of staff administering consent	Date	Signature

*If participant cannot give consent on their own e.g. mentally ill

**If both participant and guardian cannot read or write.

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Appendix 4D. Consent form 3



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Centre	Study Number	Patient Identification Number

PATIENT CONSENT FORM 3

DETERMINANTS OF DIABETIC RETINOPATHY SEVERITY AND PROGRESSION IN MALAWI

Statement by patient. Please circle Yes or No to confirm whether you agree or do not agree with each of the points below:

- I have read the information sheet or it has been read to me Yes / No
- I have had the chance to ask questions and I am satisfied with the answers Yes / No
- I voluntarily agree that my blood samples can be tested for HIV infection in the laboratory Yes / No
- I understand that the results of this test will not be disclosed to me Yes / No
- I understand that if, at a later date, I wish to know the result of the test that I have the right to request this information from the research team Yes / No

Name of participant	Date	Signature
Name of guardian	Date	Signature
Name of staff administering consent	Date	Signature